

Review

## Evaluation of colistin as an agent against multi-resistant Gram-negative bacteria

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### Abstract

Infections caused by multi-resistant Gram-negative bacteria, particularly *Pseudomonas aeruginosa*, are increasing worldwide. In patients with cystic fibrosis (CF), resistance in *P. aeruginosa* to numerous anti-pseudomonal agents is becoming common. The absence since 1995, of new substances active against resistant Gram-negative bacteria, has caused increasing concern. Colistin, an old antibiotic also known as polymyxin E, has attracted more interest recently because of its significant activity against multi-resistant *P. aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*, and the low resistance rates to it. Because its use as an anti-pseudomonal agent was displaced by the potentially less toxic aminoglycosides in 1970s, our knowledge of this drug is limited. However, there has been a significant recent increase in the data gathered on colistin, focussing on its chemistry, antibacterial activity, mechanism of action and resistance, pharmacokinetics, pharmacodynamics and new clinical application. It is likely that colistin will be an important antimicrobial option against multi-resistant Gram-negative bacteria, for some years to come.

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### 1. Introduction

The widespread resistance of microorganisms to antibiotics threatens to be a future medical disaster [1,2]. *Pseudomonas aeruginosa* is one such difficult-to-treat organism, and reports from the National Nosocomial Infections Surveillance (NNIS) in 1998 indicated that it then ranked second among the most commonly isolated Gram-negative pathogens [3–5]. Chronic pulmonary infections with *P. aeruginosa* are a major clinical problem for patients with cystic fibrosis (CF) [4,6], and a public health threat [7]. More importantly, multi-resistant *P. aeruginosa* isolated from the infected lungs of these patients have a significantly higher mutation rate than those from other clinical sources [8].

Numerous anti-pseudomonal antibiotics are used currently for the treatment of bronchial infections, includ-

ing ticarcillin, carbenicillin, piperacillin, tazobactam, tobramycin, gentamicin, amikacin, ciprofloxacin, ceftazidime, imipenem, cilastatin and aztreonam. However, resistance to these agents is becoming more prevalent [9–11]. Surveillance conducted from 1997 to 2000 in the United States showed that approximately 16% of clinical isolates of *P. aeruginosa* were resistant to at least 3 of the core anti-pseudomonals (amikacin, ceftazidime, ciprofloxacin, gentamicin, imipenem, and piperacillin) and 1% were resistant to all of these antimicrobials [12]. Outbreaks of *P. aeruginosa* resistant to most available  $\beta$ -lactams, aminoglycosides and fluoroquinolones have been reported among CF patients, as well as in burns units and cancer centres [13–16]. The annual frequency of studies examining the resistance of *P. aeruginosa* to currently used antibiotics is increasing, (Fig. 1) and this highlights the growing concerns regarding effective treatment of infections caused by this microorganism. Unfortunately, there has been no new anti-pseudomonal agent released since meropenem in 1995 and significant levels of

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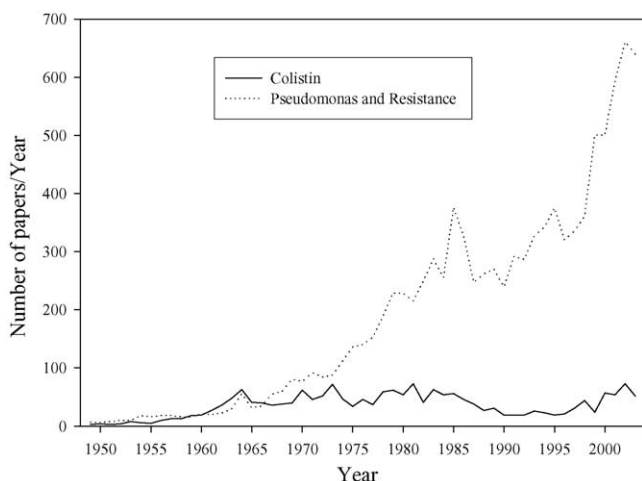


Fig. 1. Papers published containing 'colistin' vs. 'Pseudomonas' and 'Resistance' from 1949 to 2003. The results were obtained by searching Chemical Abstracts Plus with the software of SciFinder Scholar 2004 on 9 August 2004.

resistance to meropenem have already been reported in clinical isolates of *P. aeruginosa* [17].

Multi-resistance in other Gram-negative bacteria, including strains resistant to carbapenems, is also emerging as a global health issue [18,19]. Now clinical isolates with mutational fluoroquinolone resistance and metallo- $\beta$ -lactamases are being seen with increasing frequency worldwide [20]. Some species such as *Acinetobacter baumannii* strains only susceptible to polymyxins, have become a common problem especially in intensive care units [21].

Colistin, also known as polymyxin E, is an old antibiotic with significant in vitro activity against some multi-resistant Gram-negative pathogens, including *P. aeruginosa*, *A. baumannii* and *Klebsiella pneumoniae*. When the use of a  $\beta$ -lactam, aminoglycoside, or quinolone is ineffective, the polymyxins, particularly colistin, remain drugs of last resort [12]. Furthermore, resistance to colistin is seldom observed in spite of a daily selective pressure in patients receiving colistin by inhalation [22–25]. Hence, in recent years it has attracted considerable interest as an antibiotic for use against multi-resistant strains of *P. aeruginosa*, *Acinetobacter* species and *Klebsiella* species [17,24,26–29]. This trend is demonstrated in Fig. 1. The present review will focus mainly on chemical aspects of colistin, its antibacterial activity, mechanism of action and resistance, pharmacokinetics and pharmacodynamics, and recent clinical experience. Recent advances in development and validation of analytical methods for quantitation in biological fluids have enabled new insights into the pharmacokinetics and pharmacodynamics of colistin [30,31].

## 2. Discovery of colistin and early clinical experiences

Colistin is one of the polymyxin antibiotics produced by *Bacillus colistinus*. Polymyxins were discovered in 1947

[32–34]. 'Colistin', first reported by Koyama and coworkers [35], was originally thought to be distinct from polymyxins, but was later proven to be identical to polymyxin E [36]. It has been available since 1959 for the treatment of infections caused by Gram-negative bacteria [37]. However, when early clinical reports suggested a high incidence of toxicity [38,39], its use was reduced when the potentially less toxic aminoglycosides and other anti-pseudomonal agents became available. Therefore, from the decline in its use in the early 1970s up until the mid 1990s, there have been limited studies on the clinical use of colistin or on its pharmacokinetics and pharmacodynamics.

Two forms of colistin are available commercially: colistin sulphate, chiefly used topically, and sodium colistin methanesulphonate, used parenterally. Both forms may be given via inhalation. Parenteral administration of sodium colistin methanesulphonate in humans has been associated with nephrotoxicity, neurotoxicity and hypersensitivity [17,38–43]. On a weight-for-weight basis, colistin methanesulphonate is less toxic than colistin sulphate. In rats, toxicity (decreased movement, rapid respiration, etc.) was obvious after an intravenous bolus of 3.0 mg/kg colistin sulphate (in saline); however, no toxicity was observed after an intravenous bolus of 15.0 mg/kg colistin methanesulphonate in saline (unpublished data). The mechanism of toxicity at a molecular level still remains unknown. Lewis and Lewis recently demonstrated that colistin increased the transepithelial conductance in rabbit urinary bladder epithelium only when the apical membrane potential was cell interior negative [44]. This effect can be reversed over a short exposure time (<60 min at a concentration of 200  $\mu$ M). However, long exposure (120 min at a concentration of 200  $\mu$ M) produced irreversible toxicity [44].

Nephrotoxicity is one of the commonly observed adverse effects following intravenous administration of colistin methanesulphonate [24]. It usually occurs within the first 4 days of therapy, with signs continuing for 1–2 weeks after ceasing therapy; however, renal function usually returns to normal within 3–9 weeks [45]. Others have reported nephrotoxicity that was reversed as soon as therapy was discontinued in response to the first sign of developing renal impairment [24,39,40] which is consistent with the data in vitro [44]. A low dose at first followed by subsequent upward titration has been successful in decreasing the potential for toxicity [46]. However, such an approach may not be an appropriate regimen for minimizing the development of bacterial resistance. In addition, it has been suggested that inhalation of nebulized colistin sulphate by adults with CF, might cause bronchial hyper-reactivity with tightness in the chest [47]. Since a discussion of the clinical toxicity of colistin is not the main purpose of the present review, readers are referred to other reviews on the subject [17,24].

While the early clinical reports suggested a high incidence of toxicity with colistin methanesulphonate [39,42,43], closer examination of these reports has revealed an exaggerated risk which can be attributed to inappropriate selection of patients

and inadequate monitoring [17,23,40,48]. Recently, the role of colistin against *P. aeruginosa*, especially in patients with CF, has been re-examined [10,23,24,49–51]. A number of studies have confirmed the safety and efficacy of intravenous colistin methanesulphonate [41,46,48,50] and provided increased support for its use in the treatment of acute pulmonary infections due to *P. aeruginosa* [10,17,23,49,50]. Furthermore, it has been proven to be promising for the treatment of infections caused by other multi-resistant Gram-negative bacteria [29,52,53].

### 3. Chemistry

Colistin contains a mixture of D- and L-amino acids arranged as a cyclic heptapeptide ring with a tripeptide side-chain. The side-chain is covalently bound to a fatty acid via an acyl group (Fig. 2a). Sodium colistin methanesulphonate (Fig. 2b) is prepared from colistin by reaction of the free  $\gamma$ -amino groups of the Dab residues with formaldehyde followed by sodium bisulphite.

At least 30 components have been isolated from colistin and 13 identified [54–56]. They differ in the composition of amino acids and fatty acids [55–57]. Two major components are colistin A (polymyxin E<sub>1</sub>) and colistin B (polymyxin E<sub>2</sub>) (Fig. 2a). Minor components include polymyxin E<sub>3</sub> and E<sub>4</sub> [58], norvaline-polymyxin E<sub>1</sub>, valine-polymyxin E<sub>1</sub> [57], valine-polymyxin E<sub>2</sub>, isoleucine-polymyxin E<sub>1</sub>, isoleucine-polymyxin E<sub>1</sub> [59], polymyxin E<sub>7</sub> and isoleucine-polymyxin E<sub>8</sub> [55]. The proportion of colistin A and colistin B in commercial material differs between pharmaceutical suppliers and batches [60]. Colistin A has been synthesized and the

characteristic lariat structure of colistin was proven necessary for antimicrobial activity [61].

Colistin (base) exhibits both hydrophobicity attributable to the fatty acid moiety, and the basic properties ( $pK_a$  approximately 10) of the five unmasked  $\gamma$ -amino groups. Therefore, it is amphipathic and able to distribute well in both polar and non-polar environments, such as in water and in prokaryotic and eukaryotic lipid membranes. Colistin (base) is resistant to pepsin (in the pH range of 2.2–4.8), trypsin (pH 4.4–7.5), pancreatin (pH 4.4–7.5), and erepsin (pH 6.1–7.8), but is inactivated by lipase [62]. Colistin (sulphate) is less stable in water above pH 6 [63–65]. In the dry state, colistin (sulphate) is very stable at room temperature, for up to 12 months [66].

Colistin methanesulphonate hydrolyses in aqueous media and forms a complex mixture of partially sulphomethylated derivatives, with the potential to produce up to 32 different products including colistin. In 1960s, two research groups used electrophoresis and bioassay to show the appearance of various fractions from its hydrolysis in acetate buffer [67] or in human plasma and urine [67,68]. Recently, using high-performance liquid chromatography (HPLC) our group demonstrated the presence of colistin and various unidentified degradation products arising from the storage of colistin methanesulphonate in aqueous solutions [64]. McMillan and Pattison employed nuclear magnetic resonance, infrared and electrophoresis to show that the hydrolysis of the methanesulphonate group in a simple model compound, *n*-butylaminomethane-sulphonic acid involved a series of complex equilibria [69]. Unfortunately, the additional complexity introduced by the presence of five sulphomethyl groups per molecule, limited a full investigation of hydrolysis kinetics of colistin methanesulphonate. Interestingly, there have been

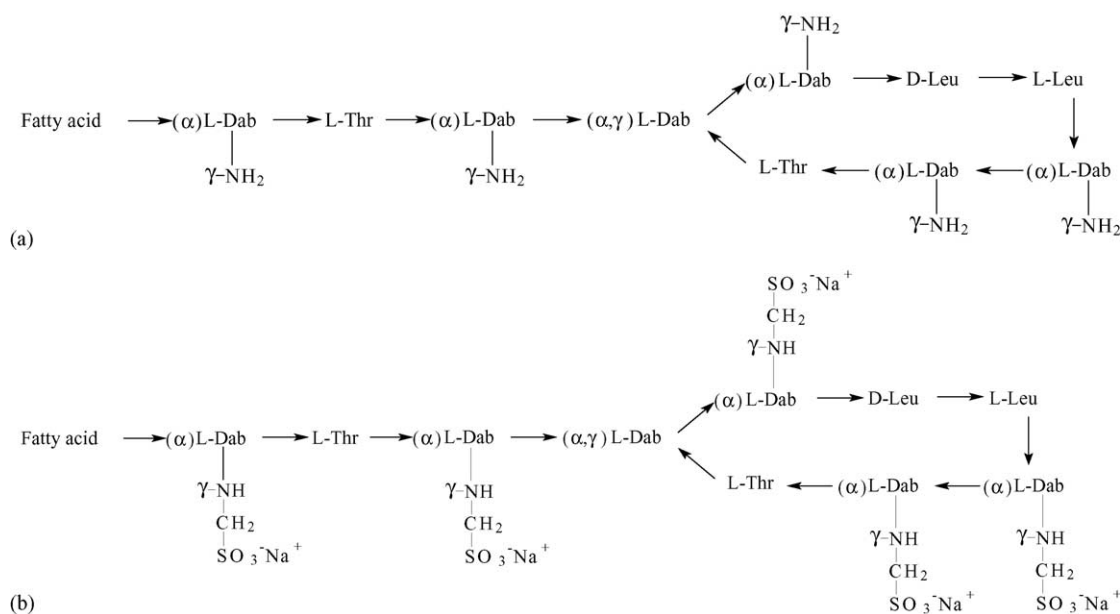


Fig. 2. (a) Structures of colistin A and B; (b) structures of sodium colistin A and B methanesulphonate. Fatty acid: 6-methyloctanoic acid for colistin A and 6-methylheptanoic acid for colistin B; Thr: threonine; Leu: leucine; Dab:  $\alpha,\gamma$ -diaminobutyric acid.  $\alpha$  and  $\gamma$  indicate the respective  $-\text{NH}_2$  involved in the peptide linkage.

reports of differences in the incidence of toxicity between the two main commercially available products of sodium colistin methanesulphonate: Colomycin<sup>®</sup> Injection (Pharmax, UK) and Coly-Mycin M Parenteral<sup>®</sup> (Parke-Davis, USA) [67]. However, HPLC analysis demonstrated that there was no colistin (base) measurable in their fresh solutions (<0.4%) and the rates of appearance of colistin (base) in plasma in vitro were comparable [64]. Given that colistin (base) is formed in vivo following the administration of colistin methanesulphonate [70], the differences in toxicity may reside in the degree of sulphomethylation during manufacture and/or storage of derivatives intermediate between colistin (base) and the fully derivatized methanesulphonate, leading to differences in the rate of formation of colistin (base) in vivo. A more rapid appearance in vivo of the more toxic colistin (base) from one of the products may contribute to its reportedly greater toxicity.

#### 4. Antibacterial activity and resistance

##### 4.1. Mechanism of action

Most investigations into the mechanism of antibacterial action of polymyxins have been conducted with polymyxin B, which is regarded as a model compound of polymyxins. Colistin, with its similar structure to polymyxin B, is believed to have an identical mechanism of action [71]. Polymyxin B interacts electrostatically with the outer membrane of Gram-negative bacteria and competitively displaces divalent cations (calcium and magnesium) from the negatively charged phosphate groups of membrane lipids [72]. Binding of polymyxin B and of colistin to the membranes can be antagonized by high concentrations of divalent cations [73,74]. Insertion of

polymyxins disrupts the outer membrane and lipopolysaccharide is released [75]. Electron microscopic results have demonstrated that membrane vesicles emerge from the surface of Gram-negative bacteria in the presence of polymyxin B [76–78]. Hancock and Chapple presented a self-promoted uptake model to explain the detailed antibacterial mechanism of cationic peptides [79]. Another characteristic of colistin which is of potential benefit is its unique anti-endotoxin activity, being able to neutralize bacterial lipopolysaccharides [80–82]. In vitro it was shown that colistin formed mixed monolayers with phospholipids and coexisted in mixed micelles [83].

The killing process with colistin is not dependent upon bacterial metabolic activity [84], and this may be a significant contributing factor towards the slow development of resistance [49], a resistance which develops more slowly than to tobramycin [23]. As observed in our laboratory [85] and by others [79], the bactericidal effect of polymyxins is extremely rapid, making it difficult to fully quantify the killing process. Masking of the five primary amine groups of colistin to form colistin methanesulphonate weakens the antibacterial activity, even after adjusting for the different molecular weights. It is presumed that the positive charged amine groups at physiological pH play an important role in the interaction with bacterial lipopolysaccharides.

##### 4.2. Spectrum of activity

Colistin exhibits a narrow antibacterial spectrum, mostly against common Gram-negative clinical isolates. Colistin is active against the common species of the Enterobacteriaceae and *Aeromonas*, but not *Vibrio* species [86], or some non-fermentative and fastidious Gram-negatives (Table 1). *Proteus* spp. [63], *Providencia* spp. [49], *Morganella mor-*

Table 1  
Susceptibility of common clinical Gram-negative bacteria against colistin

Ref	Year	Colistin form and test medium	Species	No.	MIC <sub>50</sub> (mg/L)	MIC <sub>90</sub> (mg/L)	Range (mg/L)
[96]	1965	Sulphate Methanesulphonate Brain–heart infusion agar	<i>P. aeruginosa</i>	225	3.1	12.5	0.8 to 100
			<i>P. aeruginosa</i>	225	12.5	25	3.1 to >100
[49]	1997	Methanesulphonate IsoSensitest agar	<i>P. aeruginosa</i>	94	2	4	0.5 to 32
			<i>Providencia</i> spp.	23	>128	>128	>128
			<i>Enterobacter</i> spp.	47	1	>128	0.5 to >128
			<i>Acinetobacter</i> spp.	23	1	2	1 to 128
			<i>Shigella</i> spp.	12	0.5	0.5	0.06 to 0.5
			<i>Serratia</i> spp.	24	>128	>128	16 to >128
			<i>Salmonella</i> spp.	12	1	1	1 to 2
			<i>Citrobacter</i> spp.	19	1	1	0.5 to 1
			<i>Klebsiella</i> spp.	50	1	8	0.4 to 16
			<i>E. coli</i>	50	0.5	–	0.5 to 1
[93]	2001	Sulphate Mueller–Hinton broth	<i>Acinetobacter</i> spp.	60	≤1	2	≤1 to 32
			<i>B. cepacia</i>	12	>128	>128	>128
			<i>K. pneumoniae</i>	9	≤1	≤1	≤1 to 2
			<i>P. aeruginosa</i>	80	≤1	≤1	≤1 to 2
			<i>S. maltophilia</i>	23	≤1	32	≤1 to 64
[90]	2002	Sulphate Mueller–Hinton agar	<i>P. aeruginosa</i> non-mucoid	229	1	16	≤0.12 to >128
			<i>P. aeruginosa</i> mucoid	156	0.5	2	≤0.12 to >128



*ganii* and *Serratia marcescens* [87] are resistant. *Aeromonas* species except *A. jandaei* are susceptible, although *A. hydrophila* has inducible resistance [88]. Of the common or important non-fermentative Gram-negative bacteria, *P. aeruginosa* and *Acinetobacter* species are naturally susceptible [49,89,90], *Stenotrophomonas maltophilia* are susceptible although some strains can be resistant [49,91–93]. *Burkholderia cepacia* complex [93,94] and *B. pseudomallei* [95] are resistant. Of particular importance is its activity towards multi-resistant *P. aeruginosa* [96]. *E. coli*, *Enterobacter*, *Salmonella*, *Shigella* and *Klebsiella* are also susceptible.

Colistin is also active against *Haemophilus influenzae* [89,97], *Bordetella pertussis* and *Legionella pneumophila* [98]. The pathogenic *Neisseria* spp. (including meningococci and gonococci), *Moraxella catarrhalis* [99], *Helicobacter pylori* [100,101] and *Brucella* species are naturally resistant [63]. *Campylobacter* species vary in susceptibility to colistin [102,103] and activity against *Bartonella* species is borderline [104]. Most commonly occurring Gram-positive bacteria of clinical importance are resistant to colistin.

Compared with colistin (sulphate), colistin methanesulphonate has inferior antibacterial activity [62,63,85,105,106]. The heterogeneity and potentially variable composition of colistin methanesulphonate (in terms of the degree of derivatization with methanesulphonic acid), and its instability in solution, complicate any study of its antibacterial activity. Nevertheless, our group have demonstrated recently that the fully and various partially sulphomethylated derivatives of colistin are bactericidal against *P. aeruginosa* [64,85].

#### 4.3. Susceptibility testing

Susceptibility testing methods and standards for colistin have been developed in France [107], Germany (Deutsches Institut für Normung e.V. (2000). Methoden zur Empfindlichkeitsprüfung von bakteriellen Krankheits erregern (ausser Mykobakterien) gegen Chemotherapeutika. DIN 58940. Berlin, Beuth-Verlag), and the United Kingdom [108], but not by the NCCLS in the United States [24]. Colistin sulphate is most commonly used as the test reagent, whereas the less potent colistin methanesulphonate is used clinically. As noted above, there are differences in potency between the two colistin entities, and at present it is not clear whether data from in vitro testing with the sulphate are suitable for predicting in vivo activity of the methanesulphonate. It should be noted that following administration of the methanesulphonate, it is converted, at least in part, to colistin (base) [70]. The breakpoints for susceptibility are based on colistin sulphate: the Société Française de Microbiologie has selected  $\leq 2$  mg/L as the susceptibility breakpoint, and  $> 2$  mg/L as the resistance breakpoint, the British Society for Antimicrobial Chemotherapy has selected  $\leq 4$  mg/L for susceptible and  $\geq 8$  mg/L as resistant.

Disc susceptibility testing is the most commonly recommended method for routine testing, and is described in the

French, German and UK standards. Attempts have been made to develop similar methods based on NCCLS methodology [93]. Correlation of MICs with disc diffusion zone diameters was noted to be a problem 30 years ago [109] and has been confirmed in the most recent study using NCCLS methods [93]. Around 5% of strains with elevated MICs will generate zone diameters in the 'susceptible' range. Misinterpretation of these strains as susceptible may be reduced if the advice of the SFM is followed, namely to classify as resistant any strains where colonies are detected within the zone of inhibition [107]. The reagent used in colistin disks is the sulphate salt.

#### 4.4. Acquired resistance

The potential to generate resistance to colistin is reportedly low [9,49,70,110]. However, data on acquired resistance to colistin or other polymyxins are limited. Two recent surveys have examined the prevalence of resistance in isolates of *P. aeruginosa* from CF patients [25,90]. In a single CF centre in southern Germany, 15.3% of 229 non-mucoid strains and 3.2% of 156 mucoid strains had MICs to colistin sulphate above 2 mg/L (the German resistance breakpoint) [90]. In the United Kingdom, 3.1% of 417 strains collected at multiple CF centres had MICs above 4 mg/L (the BSAC breakpoint) [25], but it was not clear whether the sulphate or the methanesulphonate was used as the test agent in that study. After more than 10 years of use in the Danish CF centre, colistin methanesulphonate has continued to demonstrate high efficacy and a low incidence of resistance [23]. However, other studies have demonstrated that resistance to colistin emerges more frequently in *P. aeruginosa* from CF patients in whom inhaled formulations have been used [85,111,112]. This refutes the frequently quoted very low potential of colistin to select for resistance, at least in *P. aeruginosa* and is notable because of the high concentrations of colistin used in inhaled formulations. Acquired resistance in species other than *P. aeruginosa* has not been well documented.

#### 4.5. Mechanisms of resistance

There are limited data on mechanisms of resistance to colistin. Studies on *P. aeruginosa* suggest a role for OprH (or H1), an outer membrane protein which is over-expressed in low  $Mg^{2+}$  environments resulting in resistance to polymyxin B and gentamicin [113,114]. In Enterobacteriaceae, changes in negatively-charged surface lipopolysaccharides induced by the regulatory loci *pmrA* and *phoP*, generate resistance to polymyxins [115].

Resistance by mutation is usually at a low level in *Salmonella* [116]. In *P. aeruginosa*, it has been proven that high-level resistance can arise from adaptation in the presence of colistin/polymyxin B in vitro [117,118]. There is cross-resistance between polymyxins [71]. However some strains of *P. aeruginosa*, while developing resistance to colistin, showed increased sensitivity to other antibiotics, such

as chloramphenicol and in particular tetracycline, to which these organisms are normally resistant [117].

At a molecular level, resistance may be due to different lipid compositions of lipopolysaccharides [118–120] or substitution of protein OprH for magnesium in the outer membrane [113,121,122]. Additional major contributors to the intrinsic multi-resistance of *P. aeruginosa* are a number of chromosomally encoded multi-drug efflux systems [123–125]. Nevertheless, colistin is not likely to be a substrate for such efflux systems, given that it interacts preferentially with the outer membrane and cytoplasmic membrane.

## 5. Clinical uses

Colistin sulphate is administered orally for the treatment of bacterial diarrhoea in infants and children and applied locally for conditions such as otitis externa and eye infections due to *P. aeruginosa* [106].

For parenteral use, colistin is administered as colistin methanesulphonate. Early experience showed it to be an effective antimicrobial agent for the treatment of septicaemias, wound infections, urinary tract infections and respiratory system infections caused by *P. aeruginosa* [24,126–128]. The predominant use over the last 20 years has been for inhalational treatment of *P. aeruginosa* infection in CF patients [22,129–132]. Inhalation doses of 1–2 million units (approximately 80 to 160 mg) of colistin methanesulphonate per day are recommended. The methanesulphonate is preferred as colistin sulphate inhalation has a high incidence of respiratory irritation [133]. The value of this approach for the prevention or delay of the onset of chronic colonization with *P. aeruginosa* and its associated effects on deterioration in lung function has been confirmed [17]. Recently, an effective system for the inhalation of colistin sulphate dry powder has been developed to overcome the problems of foaming and reduced activity following inhalation of the intravenous formulation of colistin methanesulphonate. This has allowed a shorter administration time (<1 min) [134–136]. However, the safety and tolerance of this new inhalation system is yet to be extensively investigated [17].

Recently, there has been a resurgence of interest in colistin methanesulphonate for the treatment of infections with Gram-negative bacteria resistant to other antibiotics [24,29,47]. Most attention has been focused on multi-resistant strains of *P. aeruginosa* and *A. baumannii*. Levin et al. used intravenous colistin methanesulphonate doses of 2.5–5 mg/kg per day up to a maximum of 300 mg in two or three divided doses to treat a range of infections caused by multi-resistant strains of *P. aeruginosa* and *A. baumannii* in 59 patients, including pneumonia, urinary tract infection, bacteraemias, central nervous system infection, peritonitis, catheter-related infection and otitis media [128]. In cases where infection was proven ( $n = 42$ ) rather than probable, good outcomes were observed in 67% overall, although for pneumonia a response rate of only 25% was observed [128]. Similar outcomes were observed in intensive care pa-

tients with ventilator-associated pneumonia caused by multi-resistant *A. baumannii* in a Spanish hospital [52]. Using similar doses of colistin methanesulphonate and treating strains only susceptible to colistin, the investigators cured 12 of 21 patients [52]. Outcomes, namely cure, in-hospital mortality, pneumonia-related mortality and incidence of renal failure, were similar to what they observed when patients were treated with imipenem for carbapenem-susceptible pneumonia [52]. Linden et al. showed colistin methanesulphonate to have good clinical efficacy in seriously ill patients with multi-resistant *P. aeruginosa* sepsis, curing 14 of 22 (61%) ventilated intensive care patients with septic shock and/or renal failure [28]. Similarly, Markou et al. showed a response rate of 73% (of 26 treatments) in 24 intensive care patients with multi-resistant *P. aeruginosa* and *Acinetobacter* species infections, using intravenous colistin methanesulphonate with a dose of 3 million units three times daily adjusted for creatinine clearance [53]. A single case of severe sepsis caused by *K. pneumoniae* resistant to all other drug classes and treated successfully with intravenous colistin methanesulphonate 3 million units (8.3 mg/kg) three times daily has also been reported [29], and intraventricular colistin methanesulphonate has been used to cure a case of ventriculitis caused by *A. baumannii* [137]. All the clinical studies mentioned above were reported since 1999.

Recent clinical successes with intravenous colistin methanesulphonate for respiratory exacerbations in CF patients have confirmed its efficacy and safety [41,46,48,50]. The usual recommended dose is 1.5–3 mg/kg (in 10 or 50 mL saline) administered three times daily as an intravenous infusion over 10–30 min [48].

However, current intravenous dosing regimens with colistin methanesulphonate for treating respiratory infections in CF patients have been derived from experience with its use more than two decades ago, and their appropriateness has not been fully established for treating the emerging multi-resistant Gram-negative organisms. Before this can be achieved, it is necessary to have a better understanding of the disposition and time-course of colistin methanesulphonate and colistin (base) in humans and their efficacy against these microorganisms in vivo. This is also of great importance in order to minimize the development of resistance.

## 6. Pharmacokinetics of colistin (sulphate or base) and colistin methanesulphonate

As noted above, a greater understanding of the pharmacokinetics of colistin methanesulphonate and colistin (base) in humans should offer considerable scope for improving the use of colistin for infections. However, in only two published pharmacokinetic studies, have more specific methods such as HPLC been used to measure concentrations in the plasma of humans following doses of colistin methanesulphonate [46,70]. Most data reported previously on the concentrations of 'colistin' in plasma and urine were derived using microbi-

ological assays [24]. Therefore, unless specified otherwise, the concentrations, and resultant pharmacokinetic data, discussed in Section 6.2 below were obtained from microbiological assays.

Given that colistin (base) and colistin methanesulphonate have different structures (Fig. 2), antibacterial activity [85,96] and toxicity [67,68], their pharmacokinetics are summarized separately below. However, before reviewing their respective pharmacokinetics, it is essential to review the methods that have been used for measuring their concentrations in biological fluids.

### 6.1. Methods for quantifying colistin (base or sulphate) and colistin methanesulphonate in biological fluids

#### 6.1.1. Colistin (base or sulphate)

Numerous assays for colistin (base or sulphate) have been developed based on microbiological [138], thin-layer chromatographic (TLC) [58,139], immunological [140], capillary electrophoretic [141], and HPLC methods [30,54,57,60,142–145]. With microbiological methods, *E. coli* 95 ISM, *Bordetella bronchiseptica* ATCC 4617 and other sensitive strains have most commonly been used as the test strains. *B. bronchiseptica* ATCC 4617 is particularly sensitive to colistin, more so than *E. coli* 95 ISM [62]. Unfortunately, microbiological assays lack specificity, particularly when samples contain any other co-administered antibiotics that are active against the test strain. Furthermore, microbiological methods require considerable time for incubation (up to 21 h [138]) which, given the potential lack of stability of colistin methanesulphonate in assay media, means that they are not capable of measuring the concentrations of colistin (base) in plasma and urine accurately following administration of the methanesulphonate. An immunological method has been described for measuring colistin (sulphate) in tissues from fish, but preparation of the immunogen was complex [140]. Neither of these two methods is able to quantify colistin A and B separately [140]. While TLC [58,139], capillary electrophoresis [141] and HPLC [54,57,65,142,143] have been used for the separation of the components of colistin (base or sulphate) in raw materials, only limited HPLC assays [30,60,144,145] have been extended to measuring concentrations in biological fluids.

The difficulty with analysing colistin (base or sulphate) in biological fluids by HPLC lies in its very weak ultraviolet absorption and lack of native fluorescence. Two HPLC methods for measuring colistin sulphate in biological material used *ortho*-phthalaldehyde (OPA) as the derivatizing reagent [60,144]. Unfortunately, the OPA derivatives are not very stable. Therefore, reaction conditions need to be carefully controlled and automated on-line derivatization is usually required [146]. To improve reliability, Decolin et al. employed sequential derivatization with a switching HPLC system to assay colistin in bovine milk, muscle, kidney, liver and fat tissues [60]. Le Brun et al. reported a method for measuring colistin in serum, urine and sputum, but with what appeared

to be colistin methanesulphonate as the reference standard [144].

A simple, selective and sensitive HPLC method has been developed by our group for determining colistin (base or sulphate) in plasma [30]. Reversed-phase HPLC was preceded by derivatization with fluorescent 9-fluorenylmethyl chloroformate (FMOC-Cl) in the same solid-phase extraction cartridge used to separate colistin from plasma. With this method, colistin can be measured without interference from the methanesulphonate derivatives and unlike the derivatives with OPA, those with FMOC were stable for up to 3 days at ambient temperature [30]. Furthermore, it is able to quantify colistin A and B separately [30]. This method has been employed extensively for investigating the pharmacokinetics of colistin following the administration of colistin sulphate [147] and colistin methanesulphonate in rats [148] and of colistin methanesulphonate in CF patients [70]. Recently, Gmur et al. validated an HPLC assay for colistin A in rat and dog plasma with derivatization using dansyl chloride [145]. Their sensitivity and concentration range [145] are similar to ours [30].

#### 6.1.2. Colistin methanesulphonate

Microbiological assay is the most common method currently available for quantifying colistin methanesulphonate in biological fluids [62,138]. However, colistin (base), which may arise from the hydrolysis of colistin methanesulphonate during incubation and *in vivo*, is more microbiologically active than colistin methanesulphonate [63,67,68,85,96]. In addition, colistin (base) diffuses more slowly than colistin methanesulphonate in agar [149]. Therefore, the accuracy of previously reported data on the concentrations of colistin methanesulphonate measured by microbiological methods is most likely compromised by the presence of the more microbiologically active colistin (base) and its partially sulphomethylated derivatives, in the biological samples upon collection and when formed during incubation [149]. The relative concentrations of colistin methanesulphonate and colistin (base) present in a given biological sample at the end of incubation are likely to depend on the length of incubation and will differ from the proportions present in the sample at the time of collection. Thus, reported concentrations are best regarded as 'apparent' concentrations.

To date, no HPLC method has been developed for the assay of colistin methanesulphonate in biological fluids except for the one published by our group recently [31]. This simple and sensitive method measures the summed concentrations of all sulphomethyl derivatives and colistin (base) in samples. When combined with data from a separate assay for colistin (base) [30], it is possible to determine the concentrations in biological fluids of colistin and, by difference, those of the full and partial sulphomethyl derivatives of colistin. This is a substantial improvement on the less specific microbiological methods. This method has been employed extensively for investigating the pharmacokinetics of colistin methanesulphonate in rats [148] and in CF patients [70].

Given the complexity of the hydrolytic products of colistin methanesulphonate, it will be an analytical challenge to separate and measure the concentrations of each form simultaneously with sufficient sensitivity, even with HPLC or capillary electrophoresis coupled to mass spectrometry. The only other HPLC method for measuring 'colistin' used the methanesulphonate as a reference standard and employed derivatization with dansyl chloride [46]. It was not clear which form was quantified by this HPLC method [46], colistin or colistin methanesulphonate or a combination of the two.

## 6.2. Pharmacokinetics of colistin (base or sulphate)

This section discusses the pharmacokinetics of colistin following the administration of colistin sulphate. Due to the potential for toxicity, there have been very few reports on the pharmacokinetics of colistin (base or sulphate) in humans, with most information after a parenteral dose of colistin sulphate coming from studies in dogs [150], calves [151,152] and rats [147]. It should be noted that only our recent report in rats [147] was based on concentrations measured with HPLC; the others having used microbiological methods.

### 6.2.1. Absorption

Colistin sulphate is poorly absorbed from the adult gastrointestinal tract, mucosal surfaces, inflamed surfaces or burns [47]. There was poor and variable absorption of oral doses of colistin sulphate in rabbits [63]. Interestingly, absorption from the gastrointestinal tract was observed in newborn cows (<12 h old) [153] and human infants [37]. Colistin was rapidly absorbed after intramuscular injection in calves with a serum peak at 0.5 h [152]. After six healthy volunteers and five CF patients inhaled a single dose (25 mg) of dry colistin sulphate powder, HPLC analysis revealed low peak concentrations in serum, with values ranging from 77 to 159  $\mu\text{g/L}$  [134,135].

### 6.2.2. Distribution

The volume of distribution of colistin (sulphate) in calves was  $1.30 \pm 0.29 \text{ L/kg}$  [151] and  $1.02 \pm 0.29 \text{ L/kg}$  [152], while the value in rats was lower ( $0.50 \pm 0.06 \text{ L/kg}$ ) [147]. Binding of colistin (base) to several tissue components in rabbits persisted up to 5 days after a single intramuscular injection [154–156]. The percentage of colistin unbound in plasma from dogs ranged from approximately 36–67% at concentrations between 0.5 and 12 mg/L [150], while in cows a mean of  $44.0 \pm 2.8\%$  across the range from 6.2 to 12.5 mg/L [157]. Both studies employed equilibrium dialysis at 4 °C. The percentage was much lower in plasma from cows ( $31.2 \pm 5.6\%$ ) using ultrafiltration [157]. Unfortunately, it was not clear whether non-specific binding of colistin to the ultrafiltration membrane had been excluded. In our recent report on the pharmacokinetics of colistin (sulphate) in rats [147], extensive non-specific binding (>99% at 10 mg/L) to a commonly used membrane (regenerated cellulose YM-10 from Amicon) precluded the use of ultrafiltration. With equilibrium dialysis, the fractions of colistin unbound in rat plasma were 0.44,

0.45 and 0.43 at equilibrium concentrations of 1.5, 3.4 and 6.0 mg/L, respectively [147]. Interestingly, there were significant differences between the fractions of colistin A and colistin B unbound ( $P < 0.006$ ); the values for colistin A (0.35, 0.36 and 0.36) being lower than those for colistin B (0.53, 0.52 and 0.51). The higher binding for colistin A is most likely the result of its longer chain fatty acid (6-methyloctanoic acid) compared with colistin B (6-methylheptanoic acid) (Fig. 2a), and was accompanied by a significantly greater volume of distribution for unbound colistin B in the rat [147].

### 6.2.3. Elimination

Terminal half-lives ( $t_{1/2}$ ) of colistin in calves after an intravenous dose of the sulphate (5.0 mg/kg or 25000 U/kg) were  $269 \pm 58 \text{ min}$  [151] and  $271 \pm 109 \text{ min}$  [152], respectively. However, the  $t_{1/2}$  was shorter in dogs ( $150 \pm 18 \text{ min}$ ) after an intramuscular injection (2.2 mg/kg) [150], and in rats ( $74.6 \pm 13.2 \text{ min}$ ) after an intravenous bolus (1.0 mg/kg) [147]. In patients with CF, the  $t_{1/2}$  of colistin (base) was  $251 \pm 79 \text{ min}$  after the intravenous administration of colistin methanesulphonate (1.63–3.11 mg/kg) [70].

There have been very few reports on the clearance of colistin (base or sulphate). In one of the studies described above with calves, the total body clearance (CL) was  $3.4 \pm 0.5 \text{ mL/min/kg}$  after 5.0 mg/kg colistin sulphate [151]. In our study with rats administered an intravenous bolus of 1.0 mg/kg, the CL was  $5.2 \pm 0.4 \text{ mL/min/kg}$  ( $n = 5$ ) [147]. Al-Khayyat and Aronson reported that 0.13% of a 1.1 mg/kg dose, 7.5% of a 2.2 mg/kg dose and 18.5% of a 4.4 mg/kg dose of colistin sulphate were eliminated in urine collected for up to 12 h after an intramuscular injection in dogs [150]. Similar to the 1.1 mg/kg dose above, a low urinary recovery ( $0.18 \pm 0.14\%$ ) was observed in our study with rats [147]. When comparing the renal clearance of colistin (sulphate,  $0.010 \pm 0.008 \text{ mL/min/kg}$ ) with its anticipated clearance by glomerular filtration (2.3 mL/min/kg, assuming a value for glomerular filtration rate of 5.2 ml/min/kg [158]), there must be very extensive net reabsorption from tubular urine back into blood. Furthermore, comparison of the magnitude of the non-renal clearance (essentially the same as the total body clearance) and normal hepatic blood flow in the rat (72–95 ml/min/kg [159]) indicates that colistin must have a very low hepatic extraction ratio. The fate of a large percentage of the dose of colistin (sulphate) remains unaccounted for; its metabolic fate is not well described.

With our specific HPLC assay for colistin, no difference was observed between the pharmacokinetic behaviours of colistin A and B in rats [147]. However, since there was a substantial difference in the fractions of colistin A and B unbound in plasma (Section 6.2.2), the clearances with reference to the unbound drug in plasma were significantly different [147]. Clearly, the subtle difference in structures of colistin A and B (arising from the additional  $-\text{CH}_2-$  in the fatty acid of colistin A) leads to substantial differences in not only their chromatographic behaviour [30,54,60], but also their disposition in the body [147].



### 6.3. Pharmacokinetics of colistin methanesulphonate

As noted previously, the majority of the pharmacokinetic analyses following a dose of colistin methanesulphonate have been conducted with concentrations measured in biological fluids by microbiological assays. Therefore, the disposition of 'colistin methanesulphonate' discussed below is most likely representative of a complex mixture of colistin methanesulphonate, various partial sulphomethylated derivatives plus colistin. However, our recent reports on the pharmacokinetics of colistin methanesulphonate in rats [148] and CF patients [70], represent a considerable improvement on previous work because they use analytical methods that discriminate colistin methanesulphonate from colistin (base) and do not cause hydrolysis of the methanesulphonate during sample pre-treatment [70,148].

#### 6.3.1. Absorption

As with colistin sulphate, colistin methanesulphonate is very poorly absorbed from the adult gastro-intestinal tract, urinary bladder [160], mucosal surfaces or even inflamed surfaces or burns [47]. There was poor and variable absorption after oral doses in rabbits [63]. Akin to previous observations with orally administered colistin sulphate to new-born bovines (<48 h) [153], Ross et al. noted low but variable absorption after oral doses of the methanesulphonate were given to small infants, which was in contrast to the lack of absorption by adults [37]. An intramuscular injection of colistin methanesulphonate was rapidly absorbed when administered to humans, with maximum concentrations in plasma usually reached after 1–2 h [161]. However, other workers observed considerable individual variation in the rate of absorption from an intramuscular injection in humans [62].

#### 6.3.2. Distribution

Information on the volume of distribution of colistin methanesulphonate is very limited. Recently a value of  $0.09 \pm 0.02$  L/kg in CF patients was reported from the concentrations of drug measured by the abovementioned HPLC method [46], the shortcomings of which have been discussed. With the novel HPLC assay for colistin methanesulphonate developed in our group [31], the volume of distribution of colistin methanesulphonate in CF patients was  $0.34 \pm 0.10$  L/kg after an intravenous dose (1.63–3.11 mg/kg every 8 h) at steady state [70], which is comparable to a value of  $0.30 \pm 0.06$  L/kg reported in rats dosed intravenously with 15.0 mg/kg of colistin methanesulphonate [148]. The latter value was about 60% of that observed for colistin (sulphate) in rats ( $0.50 \pm 0.06$  L/kg) [147].

The fraction of colistin methanesulphonate unbound in plasma from dogs was 0.98 at a concentration of 9.6 mg/L [150] but a fraction of  $0.66 \pm 0.04$  was unbound at concentrations of 6.2 and 12.5 mg/L in plasma from ewes [157], (both using equilibrium dialysis at 4 °C for 24 h); a lower unbound value of  $0.57 \pm 0.04$  found by the latter group using ultrafiltration [157] may be due to non-specific adsorption to

the ultrafiltration membrane. Hydrolysis of colistin methanesulphonate in plasma [64] renders it almost impossible to determine its binding accurately by equilibrium dialysis or ultracentrifugation at 37 °C, while our preliminary observation of weak non-specific binding to the membrane (YM-10 regenerated cellulose, Amicon) adds a complication to its determination by ultrafiltration [148].

An extensive study on the distribution of colistin methanesulphonate was conducted in the early 1970s after an intramuscular injection (2.5 mg/kg) into the rabbit [156]. Unbound 'colistin methanesulphonate' was detectable, 1 h after the administration until 72 h, in the liver, kidney, lung, muscle and heart, but not the brain [156]. Bound drug persisted in all of these tissues, including brain, for 72 h [156]. The high concentrations of bound drug in the brain may explain its neurotoxicity. Unbound drug present in the kidney, liver and muscle declined very slowly and was present at concentrations of more than 5 mg/kg up to 5 days after the last dose, while the unbound drug present in the brain and lung was relatively constant over this period at approximately 1.0 mg/kg [156]. The authors presumed that bound drug was located on cell membranes, which may explain not only its cumulative toxicity, but also the persistence of toxic effects for many days [156]. Given that a microbiological method was used to measure 'colistin methane sulphonate', it is very likely that the bound and unbound colistin methanesulphonate was a mixture of various hydrolysis products, including colistin.

Overall, the values for volume of distribution suggest that colistin methanesulphonate is not extensively distributed outside plasma. Its poor tissue penetration may be a consequence of their relatively high molecular weight and polarity [106]. Nevertheless, it would appear that some binding to tissues exerts a profound effect on the persistence of colistin methanesulphonate in the body. However, colistin methanesulphonate binds to tissue components to a lesser degree than colistin (base), [154,156] which may arise from a masking of the amino groups by the methanesulphonate moieties; this parallels the lower toxicity and antibacterial activity of colistin methanesulphonate.

#### 6.3.3. Elimination

Two studies from our laboratory have examined the pharmacokinetics of colistin methanesulphonate in rats [147] and CF patients [70]. After the intravenous administration of colistin methanesulphonate (1.63–3.11 mg/kg every 8 h) to CF patients at steady state, the concentrations of colistin methanesulphonate in plasma at 1 h ranged from 2.6 to 9.8 mg/L, while those at 6 h were between 0.36 and 2.5 mg/L; substantial colistin (base) was also measurable in all of the samples collected, with concentrations ranging from 1.0 to 3.1 mg/L at 1 h and from 0.23 to 1.7 mg/L at 6 h [70]. The  $t_{1/2}$  of colistin methanesulphonate ( $124 \pm 52$  min) was approximately half of that for colistin (base) formed within the body ( $251 \pm 79$  min) [70]. This is the first report on the concentrations of colistin methanesulphonate and colistin (base) in CF patients measured separately in plasma. After an intra-

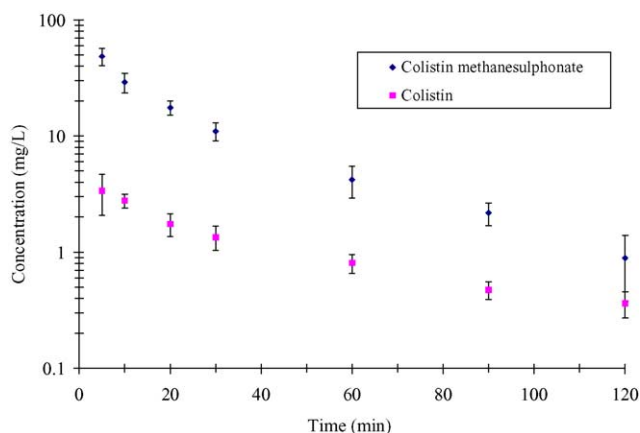


Fig. 3. Mean ( $\pm$ S.D.) plasma concentration versus time profiles for colistin methanesulphonate and colistin (base) in rats ( $n=5$ ) following an intravenous dose of the former (15.0 mg/kg) [148].

venous bolus of colistin methanesulphonate to rats, a similar relativity in the values of  $t_{1/2}$  for the methanesulphonate ( $23.6 \pm 3.9$  min) and colistin (base) ( $74.6 \pm 13.2$  min) was reported [147]. Profiles for the plasma concentrations of colistin methanesulphonate and colistin (base) versus time after an intravenous bolus of the former in rats are shown in Fig. 3 [148]. Obviously there was substantial colistin within 5 min after administration of colistin methanesulphonate. Compared with the *in vitro* stability of colistin methanesulphonate [64], it would appear that there are mechanisms other than blood/plasma-mediated hydrolysis leading to the rapid *in vivo* formation of colistin (base) [70,148].

There are several other reports on the plasma concentrations of colistin methanesulphonate after intravenous administration to humans [46,48,162] but, unfortunately, the assays were unable to discriminate colistin (base) from colistin methanesulphonate. In the report on the pharmacokinetics of colistin methanesulphonate by Reed et al. [46], the  $t_{1/2}$  was  $3.4 \pm 1.4$  h after a first dose and  $3.5 \pm 1.0$  h at steady state. In dogs after separate intramuscular injections (both at 2.2 mg/kg), concentrations measured by microbiological assays showed that colistin methanesulphonate had a shorter  $t_{1/2}$  ( $1.37 \pm 0.18$  h) than colistin (sulphate) ( $2.50 \pm 0.30$  h) [150]. Given the limitations of the HPLC method by Reed et al. [46] and microbiological assays, values for the  $t_{1/2}$  of colistin methanesulphonate in the literature, except those in our recent reports [147,148] may be composite values representing the terminal  $t_{1/2}$  of the summed concentrations of colistin methanesulphonate and colistin (base) in plasma, the latter having been formed by hydrolysis *in vivo* and during analysis.

Elimination of colistin methanesulphonate occurs mainly by the renal route [62]. A high urinary recovery of colistin methanesulphonate (approximately 60%) was reported in dogs [150] and rats [148]. Although a large proportion of the dose of colistin methanesulphonate (approximately 50%) appeared as colistin (base) in the urine in rats, it is most likely that the colistin (base) arose from hydrolysis of the methane-

sulphonate in the bladder and in the collection vessel of the metabolic cage. However, intrarenal conversion from colistin methanesulphonate cannot be excluded, and warrants further investigation. Furthermore, net renal tubular secretion of colistin methanesulphonate into the urine is apparent, which is substantially different from the very extensive tubular reabsorption observed for colistin (base) (Section 6.2.3) [147]. Clearly, derivatization of the free amine groups of colistin (base) with the methanesulphonate moiety converts the molecule from one that undergoes very extensive net tubular reabsorption to one that undergoes modest net secretion.

Similar to the studies in animals, the urinary recovery of colistin methanesulphonate was 62.5% during the first 8 h after mean doses of  $63 \pm 13$  mg were administered intravenously to patients with CF [46]. The average apparent renal clearance was  $0.24 \pm 0.15$  mL/min/kg and the mean total clearance was  $0.35 \pm 0.09$  mL/min/kg. The majority of the renal excretion (approximately 50%) occurred during the first 4 h after the dose [46]. Unfortunately urine could not be collected in our recent study on the pharmacokinetics of colistin methanesulphonate in CF patients [70]. Nevertheless, given that colistin methanesulphonate has the potential to hydrolyse in aqueous media [64] and our observed recovery of urinary colistin (base) from rats administered colistin methanesulphonate [148], it is most likely that colistin (base) would have been present in urine produced by the patients.

The fate of the remaining of colistin methanesulphonate not eliminated by the kidney in humans remains unclear [62]. Only a small part (1% to 10%) of an intramuscular dose of colistin methanesulphonate was eliminated into the faeces via bile [62,163]. In contrast, other workers have reported that intramuscularly administered colistin methanesulphonate was not excreted in the bile of humans, suggesting that biliary excretion is minor [164].

There have been few reports on the metabolism of colistin methanesulphonate in the liver or kidney. Abe et al. administered colistin methanesulphonate (100 mg/kg) intravenously to rabbits and used TLC and a reference standard to tentatively identify a metabolite of colistin, colistin-*N*-glucuronide, in the urine (1.7% of the dose) and bile (6.7% of the dose) [165].

Overall, our knowledge on colistin methanesulphonate and colistin (base or sulphate) in patients, particularly CF patients, is still limited. More clinical pharmacokinetic investigations will improve substantially our understanding of the disposition of this promising antibacterial agent. Combined with a greater knowledge of their pharmacodynamics, the useful therapeutic life span of colistin methanesulphonate and colistin sulphate can be increased.

## 7. Pharmacodynamics of colistin sulphate and colistin methanesulphonate

Apart from the considerable data on the MICs of colistin sulphate and colistin methanesulphonate (see Section 4),

very little work has been conducted on the pharmacodynamics of colistin sulphate and colistin methanesulphonate, particularly against the multi-resistant *P. aeruginosa*. Recently, the in vitro pharmacodynamic properties of colistin sulphate and colistin methanesulphonate were comprehensively investigated in our laboratory by determining the MICs, time-kill kinetics, and postantibiotic effect (PAE) against mucoid and non-mucoid strains of *P. aeruginosa* isolated from patients with CF [85]. For the susceptible strains, the MICs of colistin sulphate ranged from 1 to 4 mg/L, while the values for colistin methanesulphonate were significantly higher and ranged from 4 to 16 mg/L. Based on these findings plus other published MICs against *P. aeruginosa* [63,96], colistin sulphate is two to four times more active than colistin methanesulphonate. Unfortunately, the individual activities of colistin A and B have not been investigated.

The time-kill kinetics was explored with two clinical isolates and ATCC 27853 at concentrations ranging from 0.5 to 64× MIC. Colistin sulphate showed extremely rapid killing, resulting in complete eradication of the microorganism within 5 min at concentrations of 64× MIC; colistin methanesulphonate killed more slowly, requiring a concentration of 16× MIC to achieve complete killing within 24 h. After 15 min of exposure to the three isolates, colistin sulphate exhibited a significant PAE of between 2 and 3 h at 16× MIC. For colistin methanesulphonate, PAEs were shorter at the concentrations tested. Overall, colistin methanesulphonate had lower bactericidal and postantibiotic activities than colistin sulphate, even when adjusted for differences in MICs [85].

After allowing for the binding of colistin methanesulphonate and colistin (base) in plasma, the highest concentrations of unbound species at steady state after the administration of currently recommended intravenous doses of colistin methanesulphonate to CF patients [70] were in the same range or slightly less than the MICs observed in vitro with susceptible isolates of *P. aeruginosa* from CF patients [85]. The concentrations of colistin methanesulphonate in plasma were substantially less than the 16× MIC (64–256 mg/L) required for complete in vitro killing within 24 h and for a significant PAE. Similarly, the concentrations of colistin (base) were in a range at which *P. aeruginosa* could not be eradicated in 24 h, nor would there be a significant PAE [85]. Additionally, it is very likely that exposure to low levels of colistin sulphate and/or colistin methanesulphonate may increase the possibility of resistance developing. Hence, intravenous doses higher than 3–5 mg/kg per day in CF patients may be required to ensure the efficacy of colistin methanesulphonate. It is possible that higher doses administered less frequently, for example, once- or twice-daily, akin to the changed practice of dosing with aminoglycosides, may be a more efficacious regimen. Furthermore, given the time-dependent toxicity of colistin (base) in vitro [44] it is very likely that the toxicity may be minimized by higher doses administered less frequently. Optimized dosing regimen is being under investigation in our group.

Undoubtedly, the MICs of colistin sulphate and colistin methanesulphonate against different isolates of *P. aeruginosa* may vary over a broad range. Consequently, care is needed when comparing the MICs (determined individually for the two agents in vitro [85] and notwithstanding the potential for some hydrolysis of colistin methanesulphonate to colistin base during the incubation) with the concentrations for both agents in plasma after the administration of colistin methanesulphonate. To date, the relationship between the concentrations of colistin methanesulphonate and colistin (base) in the sputum of CF patients and therapeutic outcomes remains unclear, as does their relationships to the concentrations achieved in plasma. Thus, to decrease the possibility of resistance developing to this re-emerging antibiotic and prolong its life as a useful agent, the strategy of higher doses administered less frequently is very promising and systematic pharmacokinetic/pharmacodynamic investigations are essential.

Given its extensive urinary excretion, colistin methanesulphonate may also be a valuable antimicrobial agent for urinary tract infections caused by multi-resistant *Pseudomonas*, *E. coli* and *Klebsiella* [166]. Urine concentrations in humans measured microbiologically showed a decline from between 100 and 200 mg/L at 2 h to values ranging from 15 to 45 mg/L at 8 h after an intravenous dose of colistin methanesulphonate [162]; values well in excess of the MICs for these organisms [63]. High urinary concentrations of ‘colistin methanesulphonate’ were also observed by Reed et al. [46] and would be expected to eradicate the bacteria rapidly from the urinary tract.

Interestingly, in an experimental pneumonia model caused by multi-resistant *A. baumannii*, Montero et al. demonstrated that colistin methanesulphonate showed weaker antibacterial effect compared with imipenem, sulbactam, tobramycin, and rifampin [167]. However, in the battle against life-threatening infections due to multi-resistant *A. baumannii* worldwide, colistin has been proven, in vitro and in vivo, very promising [52,128,137,168–170]. Certainly more clinical investigations are required.

## 8. Conclusion

While colistin has been established as an effective agent against *P. aeruginosa* for several decades, its clinical use has been limited by the reported toxicities. However, much of these toxicities may be traced to its inappropriate use before the 1980s. There is an increasing appreciation of the potential value of colistin in patients infected with *P. aeruginosa*, especially with the alarming emergence of resistance to the currently available anti-pseudomonal agents. A better understanding of the pharmacokinetics and pharmacodynamics of colistin and its methanesulphonate will allow the design of appropriate dosing regimens for maximizing efficacy while minimizing toxicity and the development of resistance. Recent work in our group and in other laboratories and clinics

throughout the world has contributed significantly to a better understanding of its properties and improvements in its use. This is particularly vital for the CF patient population when they are chronically colonised with multi-resistant *P. aeruginosa*. The recent development of methods more specific for measuring concentrations of colistin (sulphate or base) and colistin methanesulphonate in biological fluids will make an important contribution to further work with this antibiotic.

After further research, it is not unreasonable to expect that colistin will be an important antimicrobial option against multi-resistant Gram-negative bacteria in the 21st century. Moreover, with developments in structure-activity relationships, combinatorial chemistry and lead optimization, the search for molecules with activity against similar microorganisms but with better pharmacokinetics, pharmacodynamics and toxicity profiles may be very profitable.

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