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REVIEW ARTICLE

Clinical Pharmacokinetics and Pharmacodynamics of Colistin

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Abstract In this review, we provide an updated summary on colistin pharmacokinetics and pharmacodynamics. Colistin is an old molecule that is frequently used as last-line treatment for infections caused by multidrug-resistant Gram-negative bacteria. Colistin is a decapeptide administered either as a prodrug, colistin methanesulfonate (CMS), when used intravenously, or as colistin sulfate when used orally. Because colistin binds to laboratory materials, many experimental issues are raised and studies on colistin can be tricky. Due to its large molecular weight and its cationic properties at physiological pH, colistin passes through physiological membranes poorly and is mainly distributed within the extracellular space. Renal clearance of colistin is very low, but the dosing regimen should be adapted to the renal function of the patient because CMS is partly eliminated by the kidney. Therapeutic drug monitoring of colistin is warranted because the pharmacokinetics of colistin are very variable, and because its therapeutic window is narrow. Resistance of bacteria to colistin is increasing worldwide in parallel to its clinical and veterinary uses and a plasmid-mediated resistance mechanism (MCR-1) was recently described in animals and humans. In vitro, bacteria develop various resistance mechanisms rapidly when exposed to colistin. The use of a loading dose might reduce the emergence of resistance but the use of colistin in combination also seems necessary.

Key Points

Because colistin binds to laboratory materials, many experimental issues are raised.

The dosing regimen of colistin methanesulfonate should be adapted to the renal function of patients, and the use of a loading dose is recommended.

Therapeutic drug monitoring of colistin is warranted.

Because the resistance of bacteria to colistin is increasing, its use in combination seems necessary.

1 Introduction

Colistin, also called polymyxin E, belongs to the group of polymyxin antibiotics (antibacterials). It is an old antibiotic discovered in the 1940s but its clinical use was largely abandoned in the 1970s mainly due to its nephrotoxicity. However, the increase of multidrug resistance (MDR) in Gram-negative bacteria (GNB), particularly *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*, led to the re-emergence of its use during the last few years [1]. Parenteral and nebulisation formulations for colistin contain the sodium salt of colistin methanesulfonate (CMS), also called colistimethate, which is an inactive prodrug. The aim of this review is to give an updated summary on colistin with respect to its complex pharmacokinetics and pharmacodynamics. A literature search was conducted using PubMed where colistin or colistin methanesulfonate were combined with key words such as "chemistry", "bioanalysis", "pharmacokinetics"

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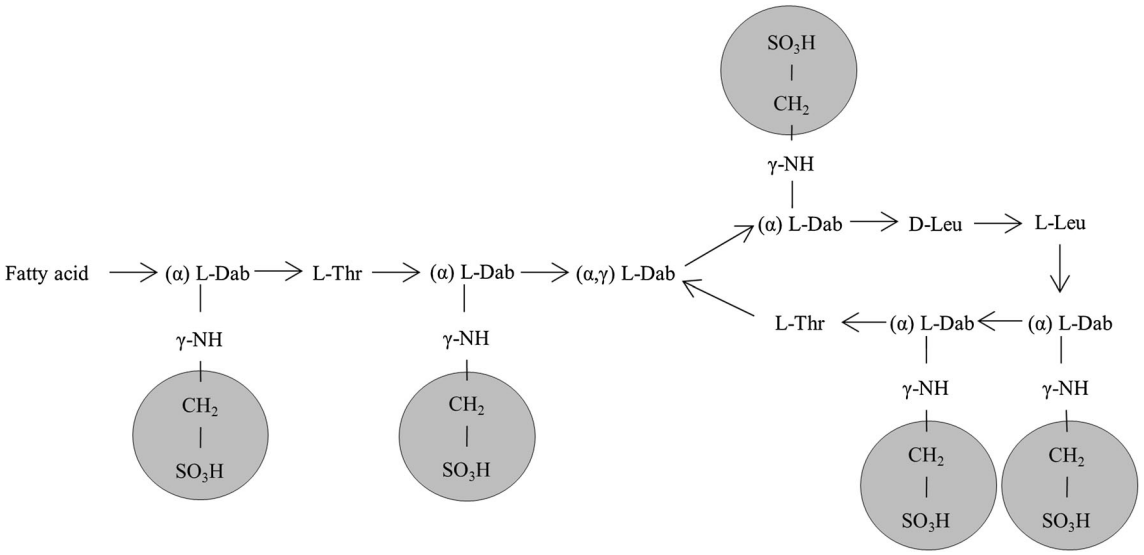

Fig. 1 Structure of colistin methanesulfonate and colistin. Sulfomethyl groups of colistin methanesulfonate are highlighted with *grey circles*. Fatty acyl: 6-methyloctanyl for colistin A and 6-methylheptanyl for colistin B; α and γ indicate the respective $-NH_2$ involved in the peptide linkage. *Dab* α ,γ-diaminobutyric acid, *Leu* leucine, *Thr* threonine

or "pharmacodynamics" to identify relevant literature. Additional references were identified from the references lists of published articles.

2 Conventions Used to Describe Doses of Colistin

At the first International Conference of Polymyxins in Prato, Italy, in 2013, it was stated that colistin doses should be referred to using international units (IU) or mg of colistin base activity (CBA) in order to avoid medication errors [2]. In Europe, India and few other countries, doses of CMS are expressed in million international units (MIU), but also in mg of CMS. By contrast, in North and South America, Southeast Asia and Australia, doses are expressed in mg CBA. In order to convert these different units, it should be known that 1 MIU is equivalent to about 30 mg of CBA, which corresponds to about 80 mg of CMS [2].

3 Chemistry

Colistin (commercially available as the sulfate salt) is a decapeptide compound, corresponding to a complex mixture of about 30 different compounds with two main components, colistin A and colistin B, the proportion of which can vary from batch to batch [3]. Colistin A and B are large molecules with molecular weights of 1169 and 1155 g/mol, respectively. They are composed of a hydrophilic cycloheptapeptide ring, a tail tripeptide moiety and a hydrophobic acyl chain tail, being one carbon shorter for colistin B than for colistin A (Fig. 1) [4]. Colistin is a hydrophilic drug ($\log P = -2.4$ [5]) but with an amphipathic property due to the presence of both lipophilic and hydrophilic groups [6]. Colistin exhibits basic properties (acid dissociation constant [p*K*a] of about 10) due to the unmasked γ-amino groups of the five L-α,γ-diaminobutyric acid (Dab) residues present in the cyclopeptide ring and tripeptide moiety (Fig. 1) [7]. Therefore, colistin is polycationic at pH 7.4 [8].

Colistin is administered parenterally as a prodrug, CMS. CMS differs from colistin by additional sulfomethyl groups on each of the five Dab residues (Fig. 1). In vivo, CMS undergoes hydrolysis to form a mixture of partially sulfomethylated derivatives that can eventually convert to colistin [9]. CMS A and B molecular weights are higher than for colistin (1635 and 1621 g/mol, respectively) due to the five additional sulfomethyl groups. CMS is more hydrophilic ($\log P = -12.1$ [10]), and is supposed to be less basic than colistin, but to our knowledge its p*K*a has not been reported yet [11, 12]. At a physiological pH of 7.4, CMS is a polyanion [1].

CMS and colistin were shown to aggregate into micelles at high concentrations in aqueous solution: their critical micelle concentrations (CMCs) were 3.5 mmol/L (5.7 g/L) and 1.5 mmol/L (1.8 g/L), respectively [13]. The conversion of CMS into colistin was much faster when the concentration of CMS was below the CMC (60% over 48 h) than when it was above the CMC (1% over 48 h) [13]. The instability of CMS at low concentrations in pharmaceutical formulations is of concern, particularly because active colistin is much more toxic than CMS [14].

4 Bioanalysis

It is important that bioanalytical methods discriminate between colistin and CMS. Yet in old studies (overall before the start of the twenty-first century), colistin concentrations were measured by microbiological assays that could not discriminate between the two components because CMS was converted into colistin during the experimental time-course. By contrast, recent methods use high-performance liquid chromatography (HPLC) or liquid chromatography–tandem mass spectrometry (LC–MS/MS) that separate CMS and colistin. Colistin concentrations are generally calculated by summing the peak areas of the major components, i.e. colistin A and B. The internal standard is generally polymyxin B [15]. The measurement of CMS concentrations implies a two-step method with firstly the quantitation of colistin alone and secondly the hydrolysis of CMS into colistin by sulphuric acid, and the quantitation of the total colistin formed [15, 16]. CMS concentration is then obtained by subtracting the concentration of colistin measured before hydrolysis to that measured after hydrolysis. This method does not allow the discrimination of the different sulfomethyl derivatives and therefore the reported CMS concentrations should be interpreted as the summed concentrations of all these derivatives. Therefore, the reported pharmacokinetic parameters for CMS may best be considered as hybrid parameters for CMS and partially sulfomethylated derivatives [17].

For measuring plasma concentration, sample preparation can include a simple protein precipitation using trichloroacetic acid and methanol [18–21] and/or a solid-phase extraction [15, 18–21]. After separation by chromatography, detection is carried out either by LC–MS/MS [15, 21] or fluorimetry after derivatisation of colistin [18, 19]. The reported limits of quantification for colistin concentration in plasma are 0.03–0.04 mg/L with LC–MS/MS [15, 21] and 0.1–0.3 mg/L by HPLC-fluorimetry [18, 19]. It is of note that for measuring colistin concentrations in broth culture medium, urine, broncho-alveolar liquid and other biological fluids (cerebrospinal fluid [CSF], peritoneal, etc.) it has been recommended to spike the samples with blank plasma in order to avoid matrix effect and colistin binding to experimental materials [15, 21, 22].

5 Mechanism of Action

Most investigations on the mechanisms of action of polymyxins were carried out with polymyxin B, but the similarities between the chemical structures of polymyxin B and colistin suggest that their mechanisms of action are identical [23]. The lipopolysaccharide (LPS) present at the surface of the outer membrane of GNB prevents the penetration of hydrophobic and/or large antibiotics (antibacterials) [24]. Due to its positive charge, colistin interacts electrostatically with the negatively charged outer membrane of GNB and competitively displaces calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions from the phosphate groups of LPS [7, 25]. Binding of colistin on the outer membrane is antagonised by divalent cations [26–28], resulting in a decreased antibacterial activity. It is of note that CMS, which differs from colistin by the addition of sulfomethyl groups masking the amines responsible for the positive charge, has a very weak antibacterial activity. Moreover, as the outer leaflet of mammalian cell membranes is charged neutral at physiological pH, colistin interacts less with mammalian cells [29]. Destabilisation of LPS leads to the disruption of the outer membrane, the loss of periplasmic and cytoplasmic contents and eventually bacterial death [24, 25, 30].

The endotoxin of GNB consists of the lipid A portion of the LPS, which can be shed by bacteria during antimicrobial therapy and can be responsible for endotoxic shock [31]. Colistin has an anti-endotoxin activity by binding to and neutralising the LPS [31–35].

Colistin also acts by several other mechanisms [36], such as an inhibition of vital respiratory enzymes (nicotinamide adenine dinucleotide [NADH]-quinone oxidoreductase) in the bacterial inner membrane [37].

6 Minimum Inhibitory Concentration Determination

For the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI), the reference method for minimum inhibitory concentration (MIC) determination of Enterobacteriaceae, *P. aeruginosa* and *Acinetobacter* spp. is the ISO (International Organization for Standardization)-standard broth microdilution [38, 39]. Cation-adjusted Mueller-Hinton broth (CAMHB) is used as the broth medium, with no additives included (in particular no polysorbate-80 or surfactants), the tray must be of plain polystyrene and not treated before use, and sulfate salt of colistin must be used [38, 39]. Addition of polysorbate-80 reduces the adsorption of colistin to polystyrene wells (see Sect. 12.1.1), but is currently not recommended by EUCAST and CLSI [40, 41]. The disc diffusion method should be avoided because colistin poorly diffuses in agar [42]. Moreover, the E-test method should be used with caution because about 50% of the results were reported to

be false when compared with the results of the broth microdilution method [41, 43].

7 Antibacterial Activity

Susceptibility breakpoints for colistin published by EUCAST are 2 mg/L for *P. aeruginosa*, *Acinetobacter* spp. and Enterobacteriaceae [44]. For now, the susceptibility breakpoint published by CLSI is 2 mg/L for both *P. aeruginosa* and *Acinetobacter* spp. (resistance if MIC $\geq$8 and 4 mg/L, respectively) [38], but this breakpoint should be revised in 2017.

Colistin is active against several GNB including *Acinetobacter* spp., *P. aeruginosa*, *Klebsiella* spp., *Enterobacter* spp., *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Yersinia* spp. and *Citrobacter* spp. [40, 45]. By contrast, colistin is inactive on Gram-positive bacteria, anaerobes and some GNB (*Proteus* spp., *Providencia* spp., *Morganella morganii*, *Serratia* spp. and *Burkholderia cepacia*) [40, 45].

8 Resistance

Increasing use of colistin has led to the emergence of colistin resistance worldwide and although resistance to colistin is generally less than 10%, colistin resistance rates are continually increasing [24]. Resistance to colistin has been described in many GNB species such as *A. baumannii*, *K. pneumoniae* and *P. aeruginosa* [24, 46].

Colistin resistance in GNB is most commonly related to LPS modifications via diverse routes, of which several involve two-component regulatory systems (TCSs) [24]. PhoPQ and PmrAB are two TCSs whose functions and regulations overlap [47]. PhoPQ and PmrAB both include a sensor kinase (PhoQ and PmrB, respectively), which senses environmental signals such as low Mg^{2+}, low pH or the presence of antimicrobial peptides. Moreover, exposure to colistin might also change the expression patterns of these TCSs [48, 49]. Activation of these sensor kinases lead to the phosphorylation of a response regulator (PhoP and PmrA, respectively), which, once phosphorylated, typically enhances their binding to promoters of regulated genes. Hence, phosphorylation of PhoP enhances the transcription of several genes, including *pmr*D, whose product binds to and stabilises PmrA in its phosphorylated state. Phosphorylation of PmrA upregulates the transcription of enzymes that are required for the addition of 4-aminoarabinose (L-ara4N) and/or ethanolamine to the lipid A component of LPS [6, 49–51]. These additions contribute to colistin resistance by reducing the negative charge of the bacterial membrane, and thereby decreasing the binding of positively charged colistin [50, 52, 53]. These adaptive mechanisms of resistance were generally of moderate level [52].

Recently, a plasmid-mediated colistin resistance mechanism MCR-1 was described for an *E. coli* strain in animals and human [54]. MCR-1 is a member of the phosphoethanolamine transferase enzyme family, with expression resulting in the addition of an ethanolamine moiety to the lipid A. Despite its relatively low level (MICs about 4–8 mg/L), this plasmid-mediated mechanism of resistance causes concern about a possible spread of colistin resistance into a range of enteric bacteria in humans and animals [55].

The phenomenon of colistin heteroresistance due to mutations in the chromosomal genes, involved in mechanisms such as lipid A biosynthesis (*lpxA*, *lpxC*, *lpxD*) or addition of L-ara4N, have also been described in *A. baumannii* and *P. aeruginosa* [53, 56–58]. This mechanism of resistance was shown to be of high level (MIC >128 mg/L) and was associated with a fitness cost for the bacteria [53, 56, 58]. Mutant strains were stable but in some patients the original susceptible isolate was able to re-emerge [53]. The reasons of this re-emergence were unclear, and could be due to the presence of a dormant persister population [59] or to the bacterial presence in locations inaccessible to colistin. Moreover, resistance was lost in one patient via the acquisition of a secondary mutation, which compensated for the fitness cost of drug resistance [53].

9 Clinical Pharmacokinetics

9.1 Plasma Concentrations in Healthy Volunteers

After a 1 h intravenous (IV) infusion of 1 MIU of CMS to healthy volunteers the CMS plasma concentrations reached a mean maximal value of 4.8 mg/L at the end of administration [60]. Thereafter, CMS concentrations declined biexponentially with a distribution half-life ($t_{½\alpha}$) of 0.5 h and a terminal half-life ($t_{½\beta}$) of 2.0 h (Fig. 2).

The time to maximal plasma concentrations (C_{max}) of the active compound colistin was 2 h after the start of the infusion (1 h after the infusion stop), and the mean colistin C_{max} was 0.83 mg/L. Colistin plasma concentrations declined monoexponentially with a $t_{½\beta}$ of 3.0 h. It is of note that as the $t_{½\beta}$ of colistin was longer than that of CMS, meaning that colistin elimination is not rate-limited by its formation.

9.2 Clearance and Metabolism

CMS was two-thirds cleared by renal excretion in healthy volunteers [60]. The renal clearance of CMS in healthy volunteers was about 100 mL/min, which was close to the

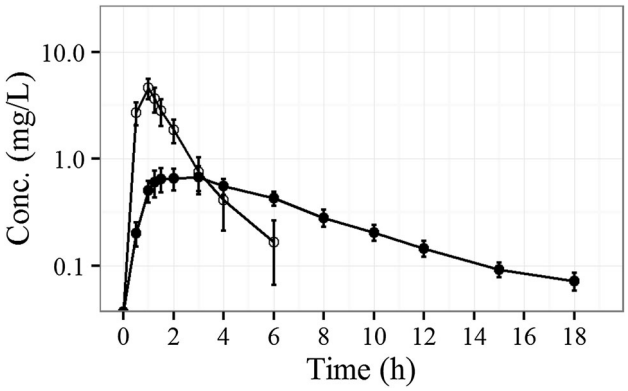

Fig. 2 Colistin methanesulfonate (*open circles*) and colistin (*filled circles*) mean (±standard deviation) plasma concentrations observed in 12 healthy volunteers after a single 1 h infusion of colistin methanesulfonate 1 MIU (million international units) (data from Couet et al. [60]). *Conc.* concentrations

glomerular filtration rate (GFR; around 120 mL/min) [60]. However, as the unbound fraction (f_u) of CMS in plasma is unknown, tubular reabsorption and secretion of CMS were not able to be estimated.

The non-renal clearance of CMS in healthy volunteers was about 50 mL/min [60]. One of the non-renal pathways for CMS clearance is its conversion into colistin by hydrolysis and removal of the five sulfomethyl groups from Dab residues. This hydrolysis leads to the formation of a series of different sulfomethylated derivatives ($2^5 = 32$ possible different derivatives) and to colistin. Other non-renal pathways, such as hydrolysis of peptide bonds, are possible for CMS but have not yet been assessed (Fig. 3).

The colistin average concentration at steady state ($C_{ss,avg}$) depends on both the fraction of CMS that is converted into colistin and the colistin clearance (adapted from Couet et al. [61]) (Eq. 1):

$$C_{ss,avg} = \frac{CL_{conv}}{CL_R + CL_{NR}} \times \frac{Dose}{\tau \times CL_{coli}}, \quad (1)$$

where CL_{conv} is the conversion clearance of CMS into colistin, CL_R is the renal clearance of CMS, CL_{NR} is the non-renal clearance of CMS (=CL_{conv} + clearance due to other non-renal pathways), τ is the dosing interval and CL_{coli} is the total clearance of colistin. As the fraction of CMS eventually converted into colistin is unknown, clearance and volume of distribution parameters for colistin are apparent parameters.

Colistin renal clearance was very low in healthy volunteers (1.9 mL/min) due to extensive tubular reabsorption [60]. The renal reabsorption of colistin may involve organic cation transporters (OCTN1), peptide transporters (PEPT2) and megalin, which is a low-density lipoprotein receptor. The renal reabsorption process is sensitive to the pH of urine [62–64]. Although renal excretion of colistin is

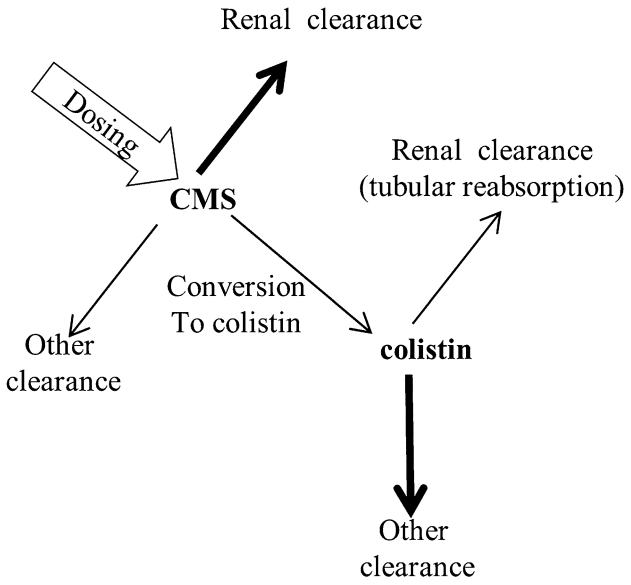

Fig. 3 Overview of the elimination pathways for colistin methanesulfonate and colistin. The *thickness* of the *arrows* indicates the relative magnitude of the respective clearance pathways when kidney function is normal. Colistin methanesulfonate includes fully and partially sulfomethylated derivatives of colistin (adapted from Nation et al. [23]). *CMS* colistin methanesulfonate

very low, urine concentrations of colistin after administration of CMS can be high because of post-excretion hydrolysis of CMS into colistin within the urinary tract.

Elimination pathways of colistin are for the most part unknown. Considering its peptidic structure, colistin should be eliminated through hydrolysis but the enzymes involved and their localisation are still unknown. Blood, liver and kidneys are likely important sites for colistin elimination because they contain large amounts of proteases and peptidases; however, due to the ubiquitous availability of these enzymes throughout the body, proteolytic degradation of colistin should not be limited to classic elimination organs [65]. It is of note that the cyclic structure of colistin helps to protect colistin from proteolytic endopeptidases and the hydrophobic acyl chain helps to protect against exopeptidases, thus explaining that the colistin half-life ($t_{½}$) is longer than that of many peptides [65].

9.3 Distribution After Systemic Administration

Due to their large molecular weights and electric charge (polyanionic for CMS and polycationic for colistin) at physiologic pH, CMS and colistin cross cellular membranes and physiological barriers poorly. Indeed, volumes of distribution of CMS and colistin in healthy volunteers (14.0 and 12.4 L, respectively, apparent volume for colistin) have been shown to be consistent with a distribution restricted to the extracellular space [60].

9.3.1 Protein Binding

Colistin has been reported to bind to α-1-acid glycoprotein (AGP), whereas the binding to other plasma components such as albumin, lipoproteins or globulins remains to be elucidated [66]. Colistin is a large molecule that cannot enter the cavity of AGP to form a tight complex and instead a two-step process of binding has been proposed [66]. An initial electrostatic attraction occurs between the positive Dab residues of colistin and the negative sialyloligosaccharides proximal to the binding cavity of AGP. The second step consists of a stabilisation of the liaison by insertion of the lipophilic tail of colistin into the hydrophobic ligand binding cavity of AGP and/or binding to lipidic substances, such as phospholipid, bound to AGP. Therefore, both the positive charge and the amphipathic properties of colistin seem necessary for its binding to AGP in plasma. The importance of the charge is exemplified by the fact that CMS, for which Dab moieties are masked, does not bind to AGP [66]. It has been shown that colistin binds to AGP with less affinity than for bacterial LPS, thus suggesting that in vivo the affinity for LPS is strong enough to dissociate and sequester the colistin from AGP [66].

Protein binding can be determined either by ultrafiltration or equilibrium dialysis [67]. However, the extensive non-specific binding (>99%) of colistin to commonly used membranes [68] requires these experiments to be implemented with specific dialysis cells and membranes [67]. The assessment of colistin protein binding using a microdialysis method raises the same problem of colistin adhesion to experimental equipment [69].

In animals, plasma protein binding of 55% ($f_u = 45\%$) has been reported for colistin in rats, dogs and calves [68, 70]. In mice, the average percentage bound was around 91% ($f_u = 9\%$) in the total concentration range of 2–50 mg/L [67].

In critically ill patients, colistin binding to plasma components was about 59–74% [71]. Across the 0.01–15 mg/L range of total concentrations, at 37 °C, the bound fraction of colistin B was constant (average 57%), whereas the bound fraction of colistin A was dependent on the concentration. At 0.1 mg/L the average binding of colistin A was 84% against 69% at 10 mg/L, meaning that the f_u of colistin A varied greatly as a function of the concentration (average 16% at 0.1 mg/L and 31% at 10 mg/L). This greater binding of colistin A, also demonstrated in rats [68], is most probably due to its longer fatty acid chain, since it is the only difference with colistin B. Whereas there is a difference in protein binding between colistin A and colistin B, to our knowledge, no difference in potency has been reported.

The level of plasma AGP can increase, depending on the disease condition, particularly bacterial infection [72]. Therefore, protein binding of colistin is expected to be higher in critically ill patients than in healthy volunteers. This has not been reported yet; however, protein binding of colistin was greater in infected mice than in healthy mice [66]. Moreover, protein binding of polymyxin B, which is chemically close to colistin, is greater in critically ill patients than in healthy volunteers [73].

In airways, colistin binds to mucin, which may reduce its antibacterial efficacy as illustrated by the >100-fold increase of MICs when mucin is added to growth medium [74].

9.3.2 Distribution Within Lung

Concentrations of CMS and colistin in epithelial lining fluid (ELF) are generally determined from concentrations measured in bronchoalveolar lavage (BAL) fluid after correction for dilution. The dilution factor is estimated based on the assumption that urea concentration is identical in plasma and ELF, by measurement of urea concentrations in plasma and BAL [22]. The determination of colistin concentration in BAL fluid should take into account the non-specific binding to the BAL material, which can be particularly high at low concentrations (80% for colistin concentrations <1 mg/L; unpublished data).

Intravenous (IV) Administration After repeated IV administrations of 2 MIU CMS every 8 h to critically ill patients, Imberti et al. [75] could not measure colistin in BAL (limit of quantification = 0.1 mg/L). By contrast, Boisson et al. [76] reported colistin concentrations in the ELF at steady state ranging between 0.1 and 29 mg/L. For their part, Yapa et al. [77] reported colistin concentrations in sputum lower than 1 mg/L after a single IV administration of CMS 5 MIU. No active transport has been reported yet for the passage of the pulmonary barrier by colistin. However, OCTN1 and PEPT2, which are involved in the renal reabsorption of colistin, are also present in the lung [78]. Moreover, the involvement of these proteins in the pulmonary transport of active substances has already been suggested: the uptake of anti-cholinergic drugs for OCTN [79] and transport of bacterial peptides for PEPT2 [80]. In addition to their capability to pass through pulmonary barrier, CMS and colistin distribution into lung also depends on their binding to lung components. This issue is not yet elucidated but it has been shown that colistin binds to mucin [74].

Inhalation Aerosolised colistin, administered as CMS, is used to treat nosocomial pneumonia caused by MDR GNB [76, 80–84]. After pulmonary administration of CMS, the presence of colistin in plasma can either result from the absorption of CMS followed by systemic conversion into colistin or from the pre-systemic conversion of CMS into colistin followed by its absorption [22]. When nebulised

directly as colistin, the absolute bioavailability was shown to be high (69%) in rats [85]. However, after nebulisation of CMS to critically ill or cystic fibrosis (CF) patients, colistin plasma concentrations were either below the limit of quantification [77] or low (<0.73 mg/L) [76, 86], except for one study for which concentrations up to 2 mg/L were reported [87]. In critically ill patients, when nebulised as CMS, only 9% of the dose reached the systemic circulation: 1.4% as colistin converted presystemically and 7.6% as CMS [76].

After aerosol delivery of CMS to critically ill patients ELF colistin concentrations were much higher than those in plasma (5- to 1000-fold) but varied considerably, from 1 to 1100 mg/L, depending on dose (1 or 2 MIU single dose or every 8 h), time (1–8 h post-dose) and the study [76, 87].

In CF patients, CMS and colistin concentrations are generally determined in sputum. In these patients, after nebulisation of single 2 or 4 MIU doses of CMS, systemic exposure to colistin was very low, whereas colistin concentrations measured in sputum ranged from 1 to 45 mg/L [77, 86]. However, a conversion of CMS to colistin during the preparation of sputum samples before bioanalytical assay cannot be ruled out in one of these studies, due to a relatively high concentration of trifluoroacetic acid used for sample preparation [86].

Overall, these studies show that colistin systemic exposure is low after CMS nebulisation, whereas colistin concentrations in lung are high.

9.3.3 Distribution Within the Central Nervous System

IV Administration The passage across the blood–brain barrier (BBB) by CMS and colistin is limited. Colistin penetration into the CSF after repeated IV administrations of CMS was demonstrated to be low (5%) in critically ill patients [88]. The presence of meningeal inflammation enhanced penetration in CSF (11%) [89]. In paediatric patients the CSF/serum ratios were reported to be 34–67% in the presence of meningitis (measurement before and after IV CMS administration), whereas it was minimal in the absence of meningeal inflammation [90]. Moreover, it has been shown in mice that LPS can induce BBB disruption by decreasing the tight junction function; this effect depends on the bacterial species and can increase colistin uptake into the brain [91]. For now, the effect of transporters on the passage of BBB by CMS or colistin has not been reported.

Intrathecal–Intraventricular Administrations Colistin concentrations in CSF are higher when patients are treated by intraventricular or intrathecal CMS administration than when they are treated intravenously. Imberti et al. [92] reported CSF concentrations continuously above 2 mg/L if the intraventricular CMS dose was greater than 0.06 MIU every 24 h. Ziaka et al. [89] reported CSF concentrations ranging between 0.6 and 1.5 mg/L when patients were treated with combined IV CMS 3 MIU every 8 h and intraventricular CMS 0.125 MIU every 24 h.

9.3.4 Distribution in Peritoneal Fluid

In one case report, after multiple administrations of CMS 2 MIU every 8 h, colistin distributed slowly into the peritoneal fluid of a patient with severe peritonitis, but colistin concentrations in peritoneal fluid were close to that in plasma at steady state [93].

9.4 Oral Route of Administration

Colistin absorption from the gastrointestinal tract is slight or absent [94]. In simulated gastric fluid it has been shown that colistin was rapidly degraded by rupture of peptide bonds in the tail tripeptide moiety under the action of pepsin [4], the formed metabolites keeping an antimicrobial activity. Colistin sulfate is sometimes used for perioperative decontamination of the digestive tract, particularly for the suppression of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E). In this indication, colistin sulfate is generally administered orally following a dosing regimen of 100 mg every 6 h, in combination with other anti-infective agents (e.g., amphotericin B, tobramycin) [94–99]. However, this practice has been shown to select colistin-resistant bacteria and its use is controversial [99–103].

9.5 Pharmacokinetics in Special Populations

9.5.1 Pharmacokinetics in Critically Ill Patients

Maintenance Dose After IV administration of CMS to critically ill patients, the pharmacokinetics of CMS was very variable (Fig. 4). The C_{max} values were observed at the end of infusion; thereafter, CMS concentrations decreased in a mono- or bi-exponential manner, with a mean $t_{½β}$ ranging from 1.9 to 4.5 h depending on the study [103–106]. The mean plasma profile was comparable to that observed in healthy volunteers, except when the renal function of the patient was altered. Indeed, concentrations of colistin are related to the renal clearance of CMS (Eq. 1), which correlates with creatinine clearance [104, 105]. For illustration, for patients with creatinine clearance values of 120, 50 and 25 mL/min, the typical renal clearances of CMS were about 100, 50 and 25 mL/min, respectively. The main impact of this decrease of CMS renal clearance when the renal function was impaired was that the fraction of CMS converted into colistin increased, e.g. 33, 50 and 67% for the three different values

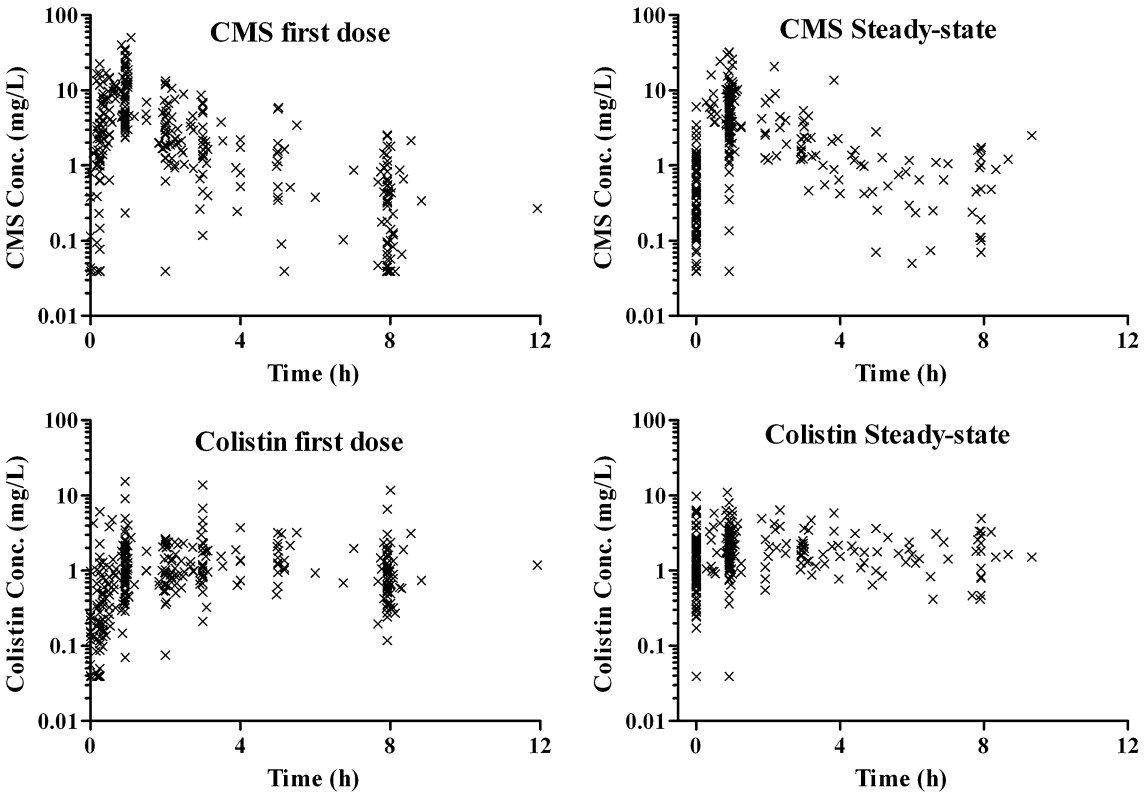

Fig. 4 Colistin methanesulfonate and colistin plasma concentrations observed (×) in 73 critically ill patients after first colistin methanesulfonate dose and at steady state. Reproduced from Grégoire et al. [105] with permission of the American Society of Microbiology. *CMS* colistin methanesulfonate, *Conc*. concentrations

of creatinine clearance of 120, 50 and 25 mL/min, thus resulting in greater colistin concentrations for patients with altered renal function. Moreover, the volume of distribution of CMS was shown to be proportional to the body weight, which after a single dose impacts on the C_{max} of CMS and colistin (to a lesser extent) and after repeated administrations impacts on the fluctuation of concentrations, i.e. the larger the volume of distribution is, the lower the concentrations fluctuate [104, 105]. However, even when considering individual renal function and body weight, the pharmacokinetics of CMS were very variable between patients after IV administration of CMS [104, 105].

Concerning the pharmacokinetics of colistin after administration of CMS to critically ill patients, some discrepancies were observed between studies. Indeed, after a first dose of 2 MIU of CMS, Grégoire et al. [105] observed typical colistin C_{max} values of about 2 mg/L, whereas after a first dose of 3 MIU of CMS Plachouras et al. [106] observed a colistin C_{max} of 0.6 mg/L [106] (Fig. 5). Moreover, the C_{max} values were reached sooner for Grégoire et al. [105] (about 3 h) than for Plachouras et al. [106] (maximum not reached at next administration, i.e. 8 h). These different time to C_{max} values were related to greater apparent volumes of distribution, resulting in longer

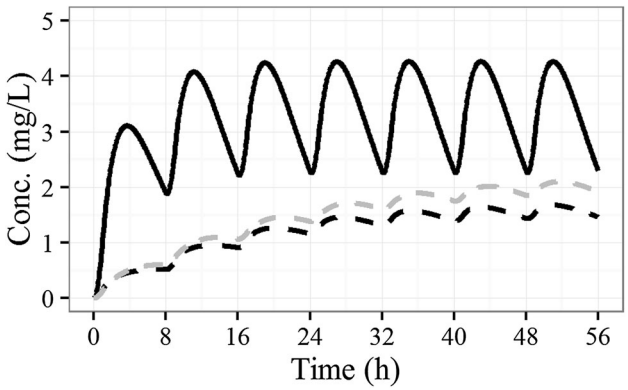

Fig. 5 Colistin concentrations following a 3 MIU (million international units) dose of colistin methanesulfonate infused over 60 min every 8 h in a critically ill patient with a creatinine clearance of 82 mL/min and a body weight of 80 kg, predicted from Grégoire et al. [105] (*solid black line*), Plachouras et al. [106] (*dashed grey line*) and Garonzik et al. [104] (*dashed black line*). Reproduced from Grégoire et al. [105] with permission of the American Society of Microbiology. *Conc*. concentrations

typical $t_{½}$ values for colistin (9–14 vs. 3 h) [103–106]. One major consequence of a longer $t_{½}$is that from Plachouras et al. [106] at least 48 h is necessary to reach steady state, whereas from Grégoire et al. [105] steady state should be reached after as soon as 12 h. At steady state there were

fewer discrepancies between studies: for a patient with a clearance of creatinine of 82 mL/min, treated with CMS 3 MIU every 8 h, the typical colistin $C_{ss,avg}$ was predicted to be between 1.5 and 3.5 mg/L depending on the study (Fig. 5) [103–106]. Plachouras et al. [106] were the first to point out the difficulties in attaining a $C_{ss,avg}$ of 2 mg/L for patients with a creatinine clearance ≥80 mL/min [106]. Recently, Nation et al. [107] suggested the use of an algorithm to calculate the CMS dose to administer according to the creatinine clearance, and demonstrated that less than 40% of the patients with creatinine clearance above 80 mL/min attained a $C_{ss,avg}$ >2 mg/L, even with a maximal 12 MIU daily dose.

Loading Dose Plachouras et al. [106] suggested that administration of a loading dose is necessary to achieve effective colistin concentrations as soon as the first CMS administration; two subsequent studies have assessed this suggestion [71, 108]. In the first study, after administration of 6 MIU of CMS to ten critically ill patients, colistin C_{max} values were on average 1.3 mg/L (range 0.3–2.6 mg/L) at 8 h following dosing and the mean colistin $t_{½}$ was 18.5 h [71]. In the second study, following administration of a 9 MIU loading dose to 19 critically ill patients the C_{max} values of colistin were also very variable (mean 2.65 mg/L, range 0.9–5.1 mg/L) and the mean colistin $t_{½}$ was 11.2 h [108]. Colistin concentrations observed in this latter study were therefore higher than expected from previous studies performed by the same team [71, 106] but lower than those predicted by Grégoire et al. [105]. Overall, these discrepancies of colistin pharmacokinetics between studies were attributed either to (1) a higher proportion of the A and B forms in the more recent CMS formulations [108]; (2) the use of different CMS brands; (3) the inclusion of patients with different renal function; (4) CMS solutions for infusions at concentrations below or above the CMCs of 5.7 g/L (71,250 IU/mL); (5) in vitro conversion of CMS to colistin after blood collection [109]; or (6) potential discrepancies in the analytical methods (e.g. in the quantification of partly sulfomethylated compounds and potential hydrolysis during work-up) [108].

Dosing Suggestions Considering the pharmacokinetics of both CMS and colistin, the CMS dose has to be adapted to each patient's renal function. In case of normal renal function, all of the previous cited studies recommend a maintenance dose of 9 MIU of CMS per day in two or three injections [105, 106], which corresponds to the calculated maintenance dose suggested by the algorithm of Garonzik et al. [104]. It is of note that the most recent publications all agree that CMS should be administered twice daily [105–108]. The maintenance dose should be adapted to the renal function of the patient. The European Medicines Agency (EMA) suggests that for patients with a creatinine clearance above 50 mL/min the daily dose should be 9 MIU (up to 12 MIU in some cases, for patients with good renal function), for patients with a creatinine clearance between 30 and 50 mL/min the daily dose should be between 5.5 and 7.5 MIU, and for patients with a creatinine clearance between 10 and 30 mL/min the daily dose should be 5 MIU [110, 111].

Concerning the loading dose, Garonzik et al. [104] suggest it should be adapted to the patient's body weight without exceeding 10 MIU, whatever their renal function, and to begin maintenance doses 24 h later. Karaiskos et al. [108] demonstrated that a loading dose of 9 MIU followed by the beginning of a maintenance dose 24 h later was safe for their 19 critically ill patients with normal renal function. The Committee for Medicinal Products for Human Use (CHMP) of the EMA proposed a loading dose of 9 MIU for patients above 60 kg and 6 MIU for patients below 60 kg; doses up to 12 MIU may be required for patients but the clinical experience with such doses is limited [110]. The loading dose should apply to all patients regardless of renal function. Recently, it was suggested that the first maintenance dose be administered 12 h after the loading dose [107].

9.5.2 *Pharmacokinetics in Patients with Haemodialysis*

In patients with highly impaired renal function, CMS is poorly excreted in urine and therefore the fraction of dose available for conversion to colistin is higher. As a consequence, on days without a haemodialysis session, colistin exposure was threefold greater in critically ill patients requiring haemodialysis than in patients with preserved renal function and treated with the same dosage [112].

Considering their molecular weights, CMS and colistin fractions unbound in plasma can freely pass through dialysis membranes. Moreover, colistin might also adsorb on dialysis membranes, notably those used for continuous renal replacement therapy, which could contribute to the removal mechanism [113]. Indeed, during intermittent haemodialysis sessions, CMS and colistin are efficiently cleared [104, 113–116]. Mean clearance of CMS during haemodialysis session was reported to be between 71 and 95 mL/min [104, 114, 116], and associated estimation of inter-individual variability was sometimes low (26% coefficient of variation) [114] but sometimes high (96%) [104]. Mean clearance of colistin during haemodialysis session was reported to be between 57 and 134 mL/min [104, 114, 116]; inter-individual variability was estimated to be moderate (15 and 44%) [104, 114].

Dosing Suggestions for Intermittent Renal Replacement Therapy Previous data suggested that during a non-haemodialysis day, the CMS daily dose should not exceed 3 MIU [104, 112], but recently Nation et al. [107] suggested administration of 3.95 MIU per day to achieve the

average steady-state colistin concentration of 2 mg/L. During a haemodialysis day, it is suggested that a supplemental dose be administered at the end of the haemodialysis session (30–50% of the daily dose) [104]. In each study, CMS was administered twice daily [104, 107, 112].

During continuous venovenous haemofiltration (CVVH) Garonzik et al. [104] reported mean CMS and colistin removal clearances slightly lower than those measured during intermittent haemodialysis (64 vs. 95 mL/min and 34 vs. 57 mL/min for CMS and colistin, respectively). During continuous venovenous haemodiafiltration (CVVHDF) Markou et al. [117] reported that extracorporeal clearance contributed to about 50% of total colistin clearance; however, the total colistin clearance was lower than that in patients with normal renal function, suggesting that a dose reduction may be needed in critically ill patients with CVVHDF. By contrast, Karvanen et al. [118] reported that colistin concentrations obtained under CVVHDF and receiving 2 MIU CMS every 8 h were lower than those for corresponding patients without CVVHDF, and consequently that CMS dosage should not be reduced for patients undergoing CVVHDF. More strongly, Karaiskos et al. [119] recommended an increased dose for patients under CVVH with a loading dose of 12 MIU of CMS followed by 13–15 MIU daily maintenance doses.

Dosing Suggestions for Patients Under Continuous Venovenous Haemofiltration The last recommendations published by Nation et al. [107] correspond with those published by Karaiskos et al. [119] and suggest a maintenance dose of 13 MIU daily divided into two doses. Concerning the loading dose, even if a loading dose of 12 MIU has been found more appropriate, clinical data of safety are limited and it is recommended not to exceed 9 MIU [107, 119]. Considering the large inter-individual variability, therapeutic drug monitoring (TDM) is advised for patients undergoing CVVHDF [120].

9.5.3 Pharmacokinetics in Cystic Fibrosis (CF) Patients

The pharmacokinetics of colistin in CF patients have been described after IV administration [77, 121] and after nebulisation of CMS. Following IV infusion of CMS, plasmatic pharmacokinetics for CMS were consistent between studies [77, 121] and with healthy volunteers [60], i.e. the reported values for clearance were about 100 mL/min, volume of distribution about 18 L and $t_{½}$ about 2.5 h. Colistin pharmacokinetics in plasma after infusion of CMS were also characterised by a $t_{½}$ close to that in healthy volunteers (4–7 h) [77, 121]. However, it should be noted that after a single IV infusion with the same CMS brand (Colimycin®), colistin (the active compound) exposure was 39% lower in CF patients than in healthy volunteers, suggesting that colistin clearance could be higher in CF patients [122].

After single nebulisation of CMS 2 or 4 MIU to CF patients, Yapa et al. [77] reported CMS and colistin concentrations in sputum that were higher (C_{max} for colistin in sputum ranging from 2.09 to 21.2 mg/L) than those resulting from IV administration ($C_{max} < 1.0$ mg/L) [77]. Moreover, the systemic availability of CMS was low (about 6%) and systemic exposures to CMS and colistin were minimal. Ratjen et al. [86] reported colistin concentrations that were significantly higher (mean C_{max} about 40 mg/L) than those reported by Yapa et al. [77] in sputum following nebulisation of a single dose of 2 MIU of CMS, but their bioanalytical method for quantitating colistin might have promoted the conversion of CMS into colistin and thus overestimate colistin concentrations in sputum.

Dosing Suggestion for CF Patients Local administration of CMS by nebulization with or without IV administration is suggested for this population. CF centres worldwide have adopted different inhaled CMS dosing regimens (dose and dosing interval), with current therapies ranging from 1 MIU of CMS twice daily to 2 MIU of CMS three times daily [122–125].

9.5.4 Pharmacokinetics in Burn Patients

In burn patients, after IV administration of CMS 5 MIU every 12 h, the typical $t_{½}$ of colistin was reported to be 6.6 h [126] and the clearance of colistin was comparable to that of critically ill patients [103–106] and healthy volunteers [60, 127], suggesting that it was not affected by the hypermetabolism in burn patients. The volume of distribution of colistin was slightly greater than that reported in healthy volunteers and either greater [105] or lower [104, 106] than those reported in critically ill patients.

10 Adverse Events

Two main types of toxicity, nephrotoxicity and neurotoxicity, are reported with the use of colistin. However, recent studies have reported that the incidence of nephrotoxicity is less common and severe than that reported in studies and case reports published until 1983 [128]. The observed nephrotoxicity was as high as 50% in old studies versus 15–25% in recent studies, although the definition of nephrotoxicity was not standardised between the studies [128]. However, in a recent study in patients with severe sepsis or septic shock, there was an incidence rate of acute kidney injury (AKI) of 44% following colistin administration [129]. Risk factors for nephrotoxicity include baseline renal impairment, age, severity of illness, nephrotoxic agents, duration of therapy and daily dose by ideal body weight [129, 130]. A residual concentration of colistin >2.42 mg/L was also reported as a predictor for

AKI [131]. In contrast, CF might be protective against the development of nephrotoxicity [130].

Colistin-associated nephrotoxicity usually occurs within the first 5 days of treatment and is reversible upon cessation of treatment [132, 133]. Renal insufficiency generally manifests as a decrease in creatinine clearance but haematuria, proteinuria, cylindruria or oliguria can also occur [128]. The nephrotoxicity of colistin is certainly related to its extensive renal tubular reabsorption due to the numerous transporters located in the proximal tubules, particularly the megalin (see Sect. 9.2) [64, 134]. Colistin toxicity could be due to its accumulation in the endoplasmic reticulum and mitochondria of renal tubular cells, resulting in a modification of the cell fate under oxidative stress [135]. Coadministration of ascorbic acid at a daily dose of 2–4 g in patients with severe sepsis was shown to reduce the AKI risk to four times lower than that in patients who did not receive ascorbic acid [129]. This effect could be explained by a double kidney-protective effect toward both colistin-induced and septic renal damage [129]. However, in another study with moderately ill patients the ascorbic acid did not offer a nephroprotective effect [134].

The incidence of neurotoxicity related to the use of colistin is lower than that of nephrotoxicity [128]. The most frequent neurological adverse effect is paraesthesia, which in old studies was reported to occur in 27 and 7.3% of patients receiving IV and intramuscular CMS, respectively. Other neurological adverse events include mental confusion, vertigo, ataxia and seizure, but the most dreaded complication is neuromuscular blockade presenting as respiratory muscle paralysis and apnoea [128, 136]. Like renal toxicity, neurological toxicity is considered to be dose dependent and is usually reversible after early discontinuation of the treatment [128].

Colistin aerosol therapy is generally well-tolerated, with few reported adverse events such as throat irritation, cough and bronchospasm [137, 138].

Intrathecal/intraventricular administration is also well-tolerated. One of the adverse effects reported is chemical meningitis (5/153 cases reported between 1972 and 2016) with complete resolution after the discontinuation of the intrathecal administrations, and there is no mention of nephrotoxicity [139].

11 Therapeutic Drug Monitoring

Because the pharmacokinetics of colistin is very variable between patients (Fig. 4), and because its therapeutic window is narrow, TDM of colistin is warranted after IV administration [140]. TDM of colistin requires a validated bioanalytical method (see Sect. 4). Because CMS can hydrolyse into colistin after sampling, it is recommended that blood specimens be drawn just before the next dose (trough), i.e. when the CMS concentrations are the lowest, and to handle the samples quickly.

It has been reported that colistin $C_{ss,avg}$ values should be higher than 2 mg/L to be effective [107]. These plasma concentrations of colistin should allow the pharmacokinetic/pharmacodynamic indices to reach target values determined in the mouse thigh infection model (ratio of the area under the unbound concentration–time curve to the MIC [*f*AUC/MIC] of about 12) for bacteria with an MIC lower than 2–4 mg/L [111], which correspond to the EUCAST breakpoint for susceptibility [44]. However, minimum plasma concentrations (C_{min}) of colistin higher than 2.5 mg/L have been associated with an increased risk of nephrotoxicity [131, 141]. Therefore, to be effective and avoid adverse events, $C_{ss,avg}$ should ideally be between 2 and 2.5 mg/L. In practice, the clinically desirable range of $C_{ss,avg}$ is rather from 2 to 4 mg/L [107], but renal function has to be monitored. Because it is preferable to draw the samples just before the next dose and because the fluctuations of plasma concentrations are relatively weak, this therapeutic window can also apply to residual concentrations. CMS dosing regimen has to be individualised according to concomitant medications and to the risk/benefit ratio for each patient.

12 Pharmacodynamics

12.1 In Vitro Pharmacodynamics

12.1.1 Experimental Issues

As the presence of Ca^{2+} and Mg^{2+} ions modifies the susceptibility of bacteria to colistin, their concentration into broth should be controlled and CAMHB is generally used for in vitro pharmacodynamic experiments with colistin [41]. From an experimental point of view, the fraction of colistin bound to CAMHB with initial colistin concentrations of 10 and 30 mg/L was 5% [142]. Therefore, the growth medium seems to not affect unbound concentrations of colistin used for in vitro experiments.

The in vitro determination of bacterial susceptibility to colistin poses numerous experimental problems. Several studies reported potential non-specific binding of colistin to experimental material [142–145]. Karvanen [146] characterised the extent of the colistin loss in different types of laboratory materials during simulated time-kill experiments without bacteria. The type of material and the concentration of colistin were the two main factors contributing to non-specific binding of colistin: out of four tested materials (glass, polypropylene, polystyrene and low protein-binding polypropylene), none performed well enough to enable to

ignore binding to material at concentrations between 0.125 and 8 mg/L. The best performing material was low protein-binding polypropylene with colistin loss between 45 and 10%. The relative loss due to binding increased when the concentration decreased; for instance in CAMHB, when using large polypropylene tubes, at 24 h the measured colistin concentration represented 13 and 62% of the 0 h concentrations of 0.125 and 4 mg/L, respectively. In polystyrene microplates the colistin losses were even larger, e.g. the measured concentration represented 4% of the expected 8 mg/L concentrations [146]. The impact of this non-specific binding on in vitro pharmacodynamic results is unclear. However, it is recommended, when possible, that low protein-binding polypropylene be used and colistin concentrations are measured during the time course of the experiments.

12.1.2 *Pharmacokinetics/Pharmacodynamics of Colistin Alone*

For colistin, in vitro pharmacokinetic/pharmacodynamic studies mainly focused on three Gram-negative pathogens: *P. aeruginosa*, *A. baumannii* and *K. pneumoniae*.

Determination of the pharmacokinetic/pharmacodynamic index that best predicted colistin efficacy on *P. aeruginosa* was performed using a dynamic in vitro pharmacokinetic model. *f*AUC/MIC was shown to be the pharmacokinetic/pharmacodynamic index that most closely correlated with the killing of *P. aeruginosa*, with target values for 2 $\log_{10}$ kill at 24 h of between 27.2 and 41.7 for reference strains (ATCC27853 and PAO1) [147].

In time-kill experiments, with a constant concentration of colistin over time, colistin was shown to be bactericidal on 21 *P. aeruginosa* strains at concentrations higher than 0.5 × MIC, with complete killing happening very quickly and bacteria becoming undetectable 4 h after treatment initiation. At concentrations equal to 0.5 × MIC, a small initial decrease in the concentration of colony forming units (cfu) was observed, followed by regrowth at 24 h [148] (Fig. 6). Time-kill experiments were also performed on the *A. baumannii* ATCC19606 reference strain and on 16 clinical isolates. Similar to what was observed with *P. aeruginosa*, low concentrations of colistin produced an initial decrease in cfu/mL followed by regrowth at 24 h [149]. In time-kill experiments on reference and clinical strains of *K. pneumoniae*, a regrowth was also observed after an initial rapid killing, even at a high colistin concentration (i.e. 64 × MIC) [150].

Dynamic in vitro models allow mimicking of human clinical regimens and evaluation of antimicrobial efficacy at concentrations varying over time. In this way, the efficacy of four different clinical dosing regimens of colistin against *A. baumannii* were compared, but none were able to eradicate the bacterial strain [151].

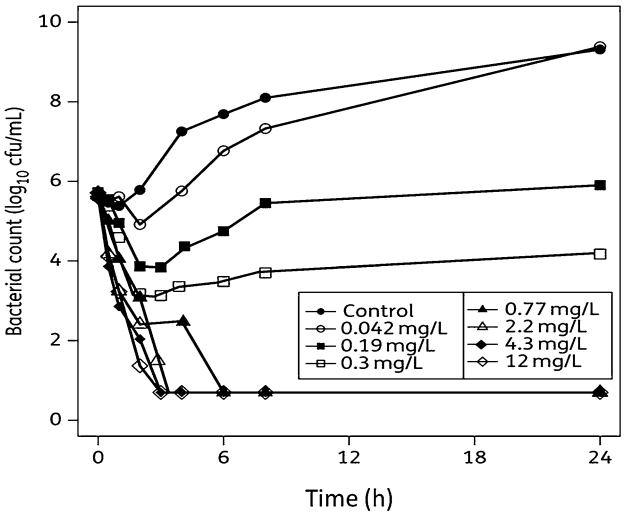

Fig. 6 Typical observed profile from one experiment for static time-kill curves for *Pseudomonas aeruginosa* exposed to colistin. Time-kill curve experiments for wild-type (ATCC27853) *P. aeruginosa* with concentrations ranging between 0.042 and 12 mg/L (minimum inhibitory concentration [MIC] = 1 mg/L). Reproduced from Mohamed et al. [155] with permission of the American Society of Microbiology. *cfu* colony-forming units

Population analysis profiles (PAPs) can be performed during time-kill or dynamic experiments in order to explore the heteroresistance phenomenon, characterised by the presence of several subpopulations of bacteria with different susceptibilities to colistin. A dynamic in vitro pharmacokinetic model was used to compare the efficacy of colistin regimens with 8, 12 or 24 h dosing intervals against *P. aeruginosa* [152]. No difference in bacterial kill was observed between regimens, but PAPs suggested that the 8 h dosing interval minimised the emergence of resistance [152]. PAPs on *A. baumannii* strains showed that 15 of 16 clinical isolates contained a resistant subpopulation, representing a small fraction of bacteria, at the start of the experiment. This so-called 'heteroresistance' was observed even though all strains were classified as colistin sensitive according to their MIC values [149]. Heteroresistance was also observed with reference and clinical *K. pneumoniae* strains, even in strains categorised as colistin sensitive based on their MIC [150].

Semi-Mechanistic Modelling Traditional analysis of pharmacokinetic/pharmacodynamic experiments are mostly qualitative, based on the variations of bacteria counts at a given time. Analysis data with semi-mechanistic mathematical models are useful to quantify the phenomenon observed during time-kill or dynamic experiments, such as bacterial resistance, antimicrobial efficacy or inoculum effect. Moreover, once a model has been developed it can be used to simulate different dosing regimens.

Table 1 Chequerboard results

Drug class	Bacteria species			References
	Klebsiella pneumoniae	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*	
β-Lactam	–	18/125	63/245	[161, 164, 165, 183, 184]
Carbapenem	3/8	1/1	63/124	[156, 158, 161, 167]
Ciprofloxacin	–	14/26	–	[183]
Fosfomycin	–	19/87	–	[157]
Glycopeptide	1/12	0/4	38/111	[157–160, 162, 169, 171, 172, 174]
Linezolid	–	–	6/40	[162]
Daptomycin	–	–	2/2	[170]
Rifampicin	8/8	–	10/45	[158, 172, 184]
Tigecycline	6/8	–	18/95	[158, 166, 184]
Trimethoprim	2/8	0/8	8/8	[174]

Fractions are given as the number of strains with a Fractional Inhibitory Concentration Index (FICI) ≤0.5/total number of strains tested

These pharmacokinetic/pharmacodynamic models have to describe the bacterial resistance to colistin. Resistance of *P. aeruginosa* and *A. baumannii* has been modelled either by splitting the bacterial population into several growing subpopulations with different susceptibility to colistin [153, 154], or with one sensitive subpopulation that adapts itself in the presence of colistin and gradually becomes resistant to it, but that can also switch to a non-growing 'persistent' form [155].

Mechanistic models can take into account some other aspects of the colistin mechanism of action, such as the inoculum effect and the competitive binding between cations (i.e. Ca^{2+} and Mg^{2+}) and colistin on the bacterial LPS [153, 154].

These models enable characterisation of the susceptibility of a specific strain to colistin (e.g. concentration of drug producing 50% of maximum effect [EC_{50}] = 1.16 mg/L for *P. aeruginosa* ATCC27853 [155]), the bacterial growth rate (e.g. mean turnover time of 75 min for *A. baumannii* ATCC19606 [154]) or the adaptation development rate (first-order adaptation rate constant of 7.2 h^{-1} for *A. baumannii* ATCC19606 [154]).

Based on a pharmacokinetic/pharmacodynamic model, recommendation of a flat fixed loading dose followed by 8- or 12-hourly maintenance doses with an infusion duration of up to 2 h was made for patients infected with *P. aeruginosa* [155]. In *A. baumannii* infection, these simulations suggested that with current regimens used in the clinical setting, polymyxin B administration was better than colistin administered as CMS because of more rapid target concentration attainment [154].

12.1.3 Pharmacokinetics/Pharmacodynamics of Colistin in Combination

In order to counteract the growing number of colistin-resistant strains, recent studies have shifted from studying colistin monotherapy to studying colistin activity in combination. Multiple methods have been used to evaluate the efficacy of combinations. Chequerboards and E-tests have been used for initial screening but, given the problems encountered with colistin E-tests (see Sect. 12.1.1), results obtained with this method are not covered in this review. Interesting combinations have been more thoroughly studied using time-kill experiments, with data resulting from these experiments being analysed with traditional methods or mathematical modelling.

Chequerboards Chequerboard results reported in the literature are summarised in Table 1. Chequerboard studies were interpreted by calculating the Fractional Inhibitory Concentration Index (FICI). Thresholds for FICI were usually as follows: FICI ≤0.5 indicated synergy, FICI between 0.5 and <4 indicated indifference/additivity and FICI ≥4 indicated antagonism [23, 155–171].

The most studied species was *A. baumannii* ($n = 670$) and the most tested antibiotic family were β-lactams ($n = 370$). From this review of the literature, the global rate of synergy between colistin and various antibiotics was 29% (280/965). When synergy was not elicited, the different combinations were at least additive or indifferent, except for some very rare cases of antagonism. Therefore, these chequerboard results support the use of colistin in combination, even if no particular antibiotic class seems to be synergistic with colistin.

Time-Kill Experiments In time-kill experiments, combinations were considered synergistic when they led at least to a 2 $\log_{10}$ cfu/mL decrease compared to the most active monotherapy at 24 h.

Colistin was shown to be synergistic with imipenem, doripenem, vancomycin, rifampicin (rifampin), trimethoprim and trimethoprim/sulfamethoxazole against *A. baumannii* strains [167, 171–174]. The addition of sulbactam improved the efficacy of the doripenem–colistin combination [173].

Against *K. pneumoniae* strains, colistin was shown to be synergistic with aztreonam, fosfomycin, meropenem, rifampicin, trimethoprim, trimethoprim/sulfamethoxazole and vancomycin [168, 174]. The same was true for aztreonam, fosfomycin and rifampicin in triple-antibiotic combinations with meropenem and colistin [168].

Colistin was shown to be synergistic with trimethoprim, trimethoprim/sulfamethoxazole and vancomycin against one colistin-resistant strain of *P. aeruginosa* [174].

Traditional time-kill criteria for evaluating synergy (e.g. $\Delta\log_{10}$ cfu/mL at 24 h) have the same limitations as FICI to show a synergy when one of the tested drugs is already effective against the studied strain, because it is hard to improve an already important effect. This could explain why synergy was more often observed against strains that were resistant to one antibiotic of the combination than against sensitive strains. Moreover, time-kill studies found synergistic combinations more often than chequerboards. This could be either because the time-kill experiments are a more powerful tool to demonstrate synergy or because time-kill experiments focused on more resistant strains. Indeed, the number of strains tested by chequerboards was generally greater than that tested by time-kill experiments because chequerboards are quicker and cheaper.

Semi-Mechanistic Modelling Built on a previously developed pharmacokinetic/pharmacodynamic model for colistin against *P. aeruginosa* [153], a model for the colistin–doripenem combination effect on *P. aeruginosa* was developed from time-kill and PAPs data [163]. In this study, multiple dosing regimens and inocula were tested. Results suggested that colistin monotherapy, even at a high dose, should be avoided due to rapid amplification of resistant subpopulations. In contrast, the results suggest that the colistin–doripenem combination would be efficient. The impact of the combination on the different subpopulations characterised by the PAPs was also assessed.

Based on another previously developed pharmacokinetic/pharmacodynamic model of colistin on *P. aeruginosa* [155], a model of the colistin–meropenem combination effect on *P. aeruginosa* was developed from time-kill data [175]. This pharmacokinetic/pharmacodynamic model suggested that the combination at clinically achievable concentrations would be efficient to treat infections with meropenem-resistant *P. aeruginosa*.

12.2 In Vivo Pharmacodynamics

Only a few in vivo pharmacodynamic studies have been performed with colistin alone or in combination. Studies of colistin monotherapy focused on the determination of the best pharmacokinetic/pharmacodynamic index and its target value. Studies of colistin combination therapy used $\Delta\log_{10}$ cfu/mL at different timepoints to assess synergism.

12.2.1 Colistin Alone

The pharmacokinetic/pharmacodynamic index that best predicted in vivo colistin efficacy was determined in mice thigh and lung infection models with three *P. aeruginosa* strains and three *A. baumannii* strains. Initially, two studies reported in vivo pharmacokinetic/pharmacodynamic index target values [176, 177], but errors in the determination of the in vivo f_u of colistin led the experiments to be repeated [67]. In this next study, *f*AUC/MIC was the pharmacokinetic/pharmacodynamic index that most closely correlated with the killing of bacteria. In the thigh infection model, target values for 2 $\log_{10}$ kill were between 7.4 and 13.7 for *P. aeruginosa* strains and between 7.4 and 17.6 for *A. baumannii* strains. In the lung infection model the target values for efficacy were much higher. It was possible to achieve 2 $\log_{10}$ kill in lung for only two-thirds of *P. aeruginosa* and one-third of *A. baumannii* strains (target values of *f*AUC/MIC between 36.8 and 105) [178].

12.2.2 Colistin in Combination

A murine thigh infection model was used to evaluate combinations of colistin and several antibiotics against extensively drug-resistant (XDR) *A. baumannii* [179] and against *K. pneumoniae* and *E. coli* [180]. Efficacy was evaluated with bacterial counts in thigh at 24 h. Rifampicin, fusidic acid and meropenem combined with colistin were synergistic against XDR *A. baumannii* [179]. By contrast, colistin and tigecycline in association were antagonist against several strains of *K. pneumoniae* and *E. coli* [180].

Colistin monotherapy and its combination with tigecycline were compared in a mice sepsis model infected by carbapenem-resistant *K. pneumoniae* [181]. Colistin and tigecycline monotherapies significantly reduced bacterial counts in liver and lung tissues, but the combination therapy was not superior to these monotherapies.

Readers especially interested in clinical combinations of polymyxins are referred to Lenhard et al. [182] for a more detailed review.

13 Conclusion

Pharmacokinetic and pharmacodynamic studies on colistin are difficult to carry out because it binds to many types of laboratory materials. Colistin renal clearance is very low due to intensive tubular reabsorption. However, the dosing regimen of colistin should be adapted to the renal function of the patient because CMS is partly eliminated by the kidney. Moreover, because the pharmacokinetics of colistin are very variable, and because its therapeutic window

is narrow, TDM of colistin is warranted. Resistance of bacteria to colistin is increasing worldwide in parallel to its clinical and veterinary uses. In vitro, when exposed to colistin, bacteria develop resistance mechanisms rapidly. In these cases, pharmacokinetic/pharmacodynamic models can be used to quantify the loss of colistin efficacy and determine optimal dosing regimens. The use of a loading dose might reduce the emergence of resistance but the use of colistin in combination also seems necessary. Some pharmacokinetic/pharmacodynamic studies of colistin in combination have already been conducted, but further investigations are necessary.

Compliance with Ethical Standards

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Conflicts of interest Nicolas Grégoire, Vincent Aranzana-Climent, Sophie Magréault, Sandrine Marchand and William Couet declare that they have no conflicts of interest.

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