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Pharmacokinetics of colistin in patients with multidrug-resistant Gram-negative infections: A pilot study

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***Background & objectives*: There is little information concerning intravenously (i.v.) administered colistin in patients with multidrug-resistant (MDR) Gram-negative infections. Thus, this pilot prospective study was undertaken to characterize efficacy and pharmacokinetics of colistin in patients with MDR Gram-negative infections.**

***Methods*: Nine patients with age >12 yr and MDR Gram-negative infections were included, of whom six were given colistin at the doses of 2 MU, while three patients were given 1 MU i.v. dose every 8 h. Blood samples were collected at different time intervals. Determination of colistin concentration was done by a ultra-high-performance liquid chromatography/mass spectrometry/selected reaction monitoring assay.**

***Results*: The area under the plasma concentration-versus-time curve over eight hours (AUC_{0-8}) for colistin after the 1st dose ranged from 3.3 to 16.4 mg×h/l (median, 4.59). After the 5th dose, AUC_{0-8} for colistin ranged from 4.4 to 15.8 mg×h/l (median, 6.0). With minimal inhibitory concentration (MIC) value of 0.125 mg/l, AUC_{0-8}/MIC ranged from 26.7 to 131.4 (median, 36.7) and 35.5 to 126.0 (median, 48.0) after the 1st and the 5th doses of 2 MU every 8 h, respectively.**

***Interpretation & conclusions*: As there is a paucity of information on AUC/MIC for colistin, it may not be possible to conclude whether AUC/MIC values in our patients were adequate. There is a microbiological clearance of organism, which goes in favour of the dosing schedule being adequate. Further studies need to be done to understand the pharmacokinetics of colistin in patients with infections.**

Key words Colistin - Gram-negative infections - MDR - pharmacodynamics - pharmacokinetics

A renewed interest in the usage of polymyxins has been observed as these are the only treatment option left for multidrug-resistant (MDR) and pan-drug-resistant pathogens such as *Acinetobacter baumannii, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*[1,2]. In recent years, *A. baumannii* has become a serious concern, especially in the Intensive Care Units (ICUs), because of the development of resistance to many antibiotics including carbapenems.

The knowledge of the pharmacokinetics (PK) and pharmacodynamics (PD) of polymyxins is

limited, resulting in inappropriate dosing, potential toxicity and development of resistance[3]. As colistin was developed six decades ago, it was not subjected to the contemporary drug development procedures. PK-PD studies of colistin methanesulphonate (CMS) and formed colistin in critically ill patients suggested modified dosing regimens of CMS in these patients[4].

CMS, an inactive prodrug form of colistin, is parenterally administered, which is a multicomponent antibiotic, with colistin A and colistin B being the two major components. It has been recognized that area under the curve/minimal inhibitory concentration (AUC/MIC) ratio is the index that best predicts the antibacterial activity, better than maximum concentration (C_{max})/MIC, suggesting that time-averaged exposure to colistin is more important than the achievement of high peak concentrations[5,6]. There is one study on Indian patients[7], which discusses about C_{max}/MIC ratio as the PK-PD index rather than the AUC/MIC. In the background of paucity of information on PK-PD of colistin in Indian patients, in particular, this pilot prospective PK-PD study was conducted on intravenously (i.v.) administered colistin in patients with MDR Gram-negative infections.

Material & Methods

This was a prospective PK-PD study of i.v. administered colistin in nine patients with MDR Gram-negative infections during 2013. The patients who were admitted to any of the ICUs of Postgraduate Institute of Medical Education and Research, Chandigarh, India, were screened for infection and, in positive cultures, identification and antimicrobial culture sensitivity were performed[8]. Patients of either sex were eligible for enrolment in the study if they were >12 yr old and had normal renal function. These patients were receiving colistin monotherapy for the treatment of bloodstream infection due to MDR Gram-negative organism susceptible only to colistin. These patients had adequate venous access to enable collection of blood for estimation of colistin in plasma.

Patients with expected survival <72 h, who were pregnant, had severe burns with >20 per cent of body surface area involvement or had cystic fibrosis were excluded from the study. Data collected included demographic information, serum creatinine and APACHE II (Acute Physiology and Chronic Health Evaluation II) score. Written informed consent was taken from the patients or their legally accepted representatives before their participation in the study, and the ethics committee of the institution approved the study (PGI/IEC/2013/1993-94).

Colistin methanesulphonate (CMS) administration: CMS was administered i.v. as a short-term infusion (for one hour) every 8 h. The recommended systemic dose of CMS for a person weighing 60 kg was given as 4-6 mg/kg/day or 1-2 million IU every eight hours at the discretion of the treating physician. The mean duration of therapy given in these patients was 13 days (13-18 days).

Pharmacokinetic sampling: Venous blood samples (3 ml) were collected from the patients in heparinized vials at the following time intervals: before the start of the first dose of colistin infusion (0 hour or baseline sample), immediately after the completion of the one-hour infusion and then at 30 min, 1.5, two, four, six, and eight hours (just after the end of the first and fourth infusions). The same schedule of blood sample collection was followed after steady state was achieved after the 5th dose. Plasma was separated from the collected blood samples and stored at −80°C till analysis of drug levels. MICs for colistin were determined by *E*-test (BioMe´rieux SA, France) as per the manufacturer's instructions.

Determination of colistin methanesulfonate and colistin concentrations in plasma: A highly sensitive ultra-high-performance liquid chromatography/mass spectrometry/selected reaction monitoring [UHPLC-MS/SRM, TSQ Vatage (Thermo fisher scientific, USA) and Agilent 1290 infinity series] assay was developed (by authors) and validated to quantify colistin A and B (colistin) from human plasma using reserpine as an internal standard. To determine the total amount of colistin, complete hydrolysis of CMS to colistin was undertaken under acidic condition (5% formic acid, 30 min). The recovery of colistin A and B (from CS and CMS) from plasma was >80 per cent. Small volume of patient plasma (200 µl) was used for colistin estimation. Both colistin A and B were well resolved on a C-18 column (first at 6.1 min and at 6.5 min, respectively, for colistin B and A). A linear relationship across a concentration range (colistin A: 0.03-2.4 µg/ml and colistin B: 0.05-3.8 µg/ml) was obtained. The regression coefficients were higher than 0.995 and accuracies were between 91 and 117 per cent with low coefficients of variation (1.5-4.4%). The dynamic range for the colistin (CMS hydrolysis) method was as follows: colistin A: 0.03-2.4 µg/ml and colistin B: 0.07-4.8 µg/ml. The regression coefficients

were higher than 0.999 and accuracies were between 99 and 104 per cent with low coefficients of variation (1.1-7.6%).

Statistical analysis: The pharmacodynamic index (AUC/MIC) was assessed with logistic regression analysis. Statistical analysis was performed using GraphPad PRISM version 6 (GraphPad Inc., La Jolla, USA).

Results

A total of 12 patients were initially selected. However, for analysis purpose, data could be included for nine patients that included eight males and one female. Of these nine patients, six patients were given colistin at the doses of 2 MU, while three patients were given 1 MU every 8 h by i.v. route as decided by the respective treating physicians. For the patients receiving 2 MU dose, the median (range) APACHE II score was 11.5 (2-26) and median (range) of (creatinine clearance) CLcr was 146.1 (70.8-195.7) ml/min. The Table describes the demographic and PK-PD details of the individual patients.

Pharmacokinetic parameters: A total of 144 samples from nine patients were available for the estimation of colistin concentrations, and pharmacokinetic parameters were analyzed after the omission of apparent outliers[9]. There was substantial inter-patient variability in the plasma concentrations of colistin achieved from the empirically selected CMS dosage regimens. However, it was observed more after the 1st dose than after the 5th dose when the steady-state levels were achieved (Figure). The volume of distribution (V_d) in the patients included in our study was as follows: median (range): 1.65 (0.34-2.99) and 1.05 (0.50-1.71) l/kg after both the 1st dose and at steady-state after 2 MU doses and 0.46 (0.13-1.7) and 1.01 (0.38-1.10) l/kg in 1 MU dose, respectively. The terminal median half-lives for colistin were 7.09 h (4.7-9.84) and 4.51 h (2.72-8.5) after the 1st and the 5th doses, respectively. The area under the plasma concentration-versus-time curve over eight hours (AUC_{0-8}) for colistin after the 1st dose ranged from 3.3 to 16.4 mg×h/l (median, 4.59). After the 5th dose, AUC_{0-8} was slightly increased and ranged from 4.4 to 15.8 mg×h/l (median, 6.0) (Table). In three patients, after 1 MU dose, AUC_{0-8} for colistin ranged from 1.9 to 6.6 (median, 4.4) and 3.5-8.5 mg×h/l (median, 5.4) for the 1st and 5th doses, respectively. AUC_{0-8}/MICs ranged from 26.7 to 131.4 (median, 36.7) and 35.5-126.0 (median, 48.0) after the 1st and the 5th doses of 2 MU, respectively.

Table. Demographics and pharmacokinetics - pharmacodynamic parameters

Patient ID	Age (yr)	Sex	BW (kg)	Diagnosis	Sample	Organism isolated	Dose MU/day	MIC mg/l	Creatinine clearance ml/min	APACHE II scoring	AUC after 1st dose mg × h/l	AUC after 5th dose mg × h/l	Outcome
1	45	Male	59	ARDS with sepsis	ETA	*Acinetobacter baumannii*	1	0.125	81.9	7	4.4	8.5	Recovered
2	28	Male	50	ARDS	ETA	*A. baumannii*	2	0.125	131.6	10	3.8	6.0	Recovered
3	55	Male	66	COPD with AE	ETA	*A. baumannii*	2	0.25	70.8	13	3.6	4.8	Recovered
4	30	Male	50	RTA with BTC	Blood	*A. baumannii*	2	0.125	99.1	2	3.3	6.0	Expired
5	45	Male	58	RTA with HI	Blood	*A. baumannii*	1	0.125	94.4	6	1.9	3.5	Recovered
6	23	Female	55	Insulinoma with HIE	ETA	*A. baumannii*	2	0.125	194.5	26	5.4	4.4	Expired
7	50	Male	90	RTA with fracture fibia	Intra-op	*A. baumannii*	2	0.125	160.6	3	5.5	11.2	Recovered
8	56	Male	70	RTA with tetanus	Blood	*A. baumannii*	2	0.25	148.5	11	16.4	15.8	Recovered
9	30	Male	60	Acute pancreatitis	Blood	*Klebsiella pneumoniae*	1	0.125	203.5	13	6.6	5.4	Expired

ARDS, acute respiratory distress syndrome; COPD, chronic obstructive pulmonary disease; AE, acute exacerbation; RTA, road traffic accident; BTC, blunt trauma chest; HI, head injury; HIE, hypoxic ischaemic encephalopathy; ETA, endotracheal aspirate; BW, body weight; APACHE II, Acute Physiology and Chronic Health Evaluation II; AUC, area under curve; MIC, minimal inhibitory concentration; MU, million International Unit

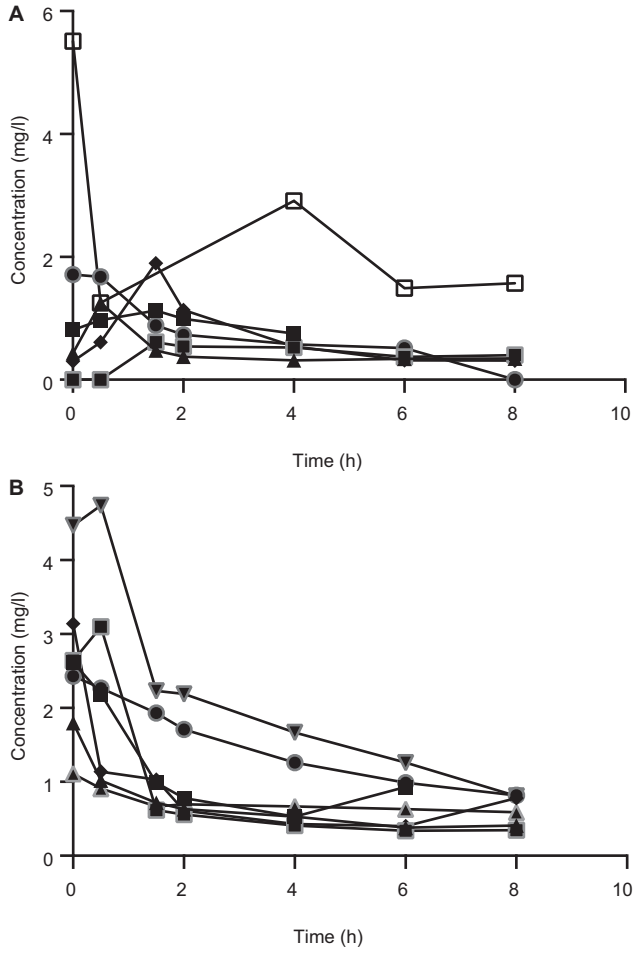

Figure. Time concentration curves of colistin after the 1st (**A**) and 5th doses (**B**) in six of nine patients who were given 2 MU three times a day.

Bacteriological profile and outcome: All the follow up cultures were sterile (Table). While 70 per cent of the patients recovered without any serious outcomes, 30 per cent did not survive. There was no significant relationship between bacteriological and clinical cures and AUC/MIC ratio, although the trend was positive (*P*=0.288 for AUC/MIC for colistin after the 5th dose with logistic regression).

Discussion

There is scarcity of data on optimized dosage of colistin, which is further complicated by inter-patient variability in same/different populations. In Indian patients, only one study is available; in which C_{max}/MIC was presented[7]. In the present study the results are described corresponding to AUC/MIC, which has been recognized as the main PK-PD index. It should be noted that the dosage of colistin used in these patients was low (1-2 MU q8h) and without a loading dose, and only patients with normal serum creatinine levels were included.

The average $t_{1/2}$ was similar to that found in other studies. Here, $t_{1/2}$ for colistin was 7.1 (4.7-9.8) h and 4.5 (2.7-8.5) h after the 1st and the 5th doses of 2 MU, respectively. With the 2 MU dosage regimen, at one hour after the start of the infusion, the apparent terminal $t_{1/2}$ was 5.9±2.6 h in a study by Imberti *et al*[10], and after 2.8 MU dosage, it was 7.4±1.7 h in another study by Markou *et al*[11], whereas other studies have reported $t_{1/2}$ estimates ranging from 9 to 18 h[12-15]. As stated by Gregoire *et al*[16], this difference in half-lives seems to arise primarily from a difference in the estimated apparent V_d. Even with the 1 MU dose regimen in our patients, $t_{1/2}$ was on higher side, which was 5.4 (3.4-16.3) and 4.4 (2.6-6.4) after the 1st and the 5th doses, respectively. Our patients had higher V_d as compared to other Indian study where it was 0.3 (0.2-0.5) l/kg after both the single dose and at steady state[7], which was higher as compared to cystic fibrosis patients [0.09 (0.02) l/kg vs. 0.09 (0.03) l/kg, respectively][17] . The average $t_{1/2}$ in our study ranged from 4.5 to 7.1 h for the two dose levels. Half-lives reported in the various studies have ranged from 5.9 to 18 h[12-15]. This wide range has been attributed to both differences in volume of distribution and/or differences in clearance. It would be expected that the volume of distribution is high in patients with sepsis with a drug like colistin. The values of V_d obtained in our study for a majority of patients were more than 1.0 l/kg. In other studies, the V_d has been reported to be 1.5±1.1 l/kg[10]. Considering the fact that we excluded patients with renal failure, clearance is not likely to have been affected.

The average steady-state concentration (*C*ss, avg) of formed colistin achieved by each of these regimens was compared to a 'target' plasma colistin *C*ss, avg of 2.5 mg/l[14]. *C*ss, avg across all six patients receiving 2 MU colistin after the 1st and 5th doses was low (median, 0.57 and 0.72 mg/l, respectively), one of the reasons being that our patients were not given the loading dose and the dose given was 2 MU q8h.

For organisms with an MIC of 2 µg/l (the current susceptibility breakpoint for *Enterobacteriaceae* defined by the European Committee on Antimicrobial Susceptibility Testing EUCAST)[18], the levels achieved in this study were inadequate for the minimal kill, indicating that the dose used was low. Using an MIC value of 2 mg/l, AUC_{0-8}/MIC ranged from 1.67 to

12.7 (median, 2.5) after the 1st dose, far below the pharmacodynamic target and similar to the values obtained by Imberti *et al*[10]. These authors also concluded that the sub-optimal dosages were given. Still, for a proportion of patients, lower exposures appear to be sufficient, both in our study as well as in others[19,20]. However, because of the low number of patients, it is difficult to draw any conclusions.

Similar to the other studies[7,13-15,21], there was substantial inter-patient variability observed in the plasma concentrations of colistin more after the first dose, which was unrelated to the creatinine clearance and the dosage being given (1 MU in three patients). The recommended systemic dose of CMS for a person weighing 60 kg used to be 4-6 mg/kg/day or 1-2 million IU/day q8h. However, the current recommendation is 4.5 million IU/day q12h[22].

The main limitation of this study was that the CMS levels were not estimated in this study as CMS is an unstable compound and ideally, it needs to be estimated in the stored samples within four months time period to avoid conversion of CMS to colistin, while these samples could be processed later than that and the median duration of sample storage prior to analysis was 11 months[17]. In addition, plasma protein binding was not measured for colistin; therefore, AUC/MIC values were for total colistin in this study. Another concern was estimation of MIC by *E*-test, which was conducted in these isolates with its limitations in the estimation of MIC, as recently noted by the EUCAST[18].

With the AUC/MIC values obtained from our study results, it will not be possible to conclusively say whether these were adequate. There is a microbiological clearance of organism, which goes in favour of the dosing schedule being adequate. However, we have been observing rising MIC values of *A. baumannii* for colistin (unpublished data) and it may be difficult to say whether our findings can be applied to this changing situation. So far, the AUC/MIC values have not been adequately defined for colistin when used for *A. baumannii*.

Based on our findings it can be presumed that as more than 50 per cent of the patients responded at the given doses (1-2 million IU/day q8h), it remains to be seen in larger studies whether smaller doses may be adequate in our patient population. In addition, in the future study, one needs to take into account the inter-patient variability observed more with colistin than with polymyxin B. In the given scenario when not much information is available in Indian patients, such studies are direly needed in view of the increasing MICs of various Gram-negative pathogenes in particular.

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***Conflicts of Interest*:** None.

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