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The pharmacokinetic-nephrotoxicity relationships of CMS and CMS-E2 from the perspective of plasma and kidney drug concentrations in rats

Chenxue Guo[1,2,3,5], Lin Xi[1,2,3,5], Xingyi Qu[1,2,3], Zhiwei Huang[1,2,3], Size Li[1,2,3], Wanzhen Li[1,2,3], Xiaofen Liu[1,2,3]✉ & Jing Zhang[1,2,3,4]✉

Nephrotoxicity has seriously affected the clinical application of colistin methanesulphonate (CMS). Colistin B methanesulphonate (CMS-E2) is a novel polymyxin developed and aimed to have lower nephrotoxicity. This study aimed to investigate the relationships between pharmacokinetics (PK) and nephrotoxicity of CMS and CMS-E2 and compare the toxicity of the two drugs in rats. Rats were treated intraperitoneally with a single dose of saline, CMS [10, 20 mg/kg of colistin base activity (CBA)], and CMS-E2 (20, 40 mg/kg CBA). An LC-MS/MS method was developed to determine plasma and renal tissue concentrations of CMS/CMS-E2 and colistin/colistin B. The severity of renal injuries was examined both biochemically and histologically. The PK-toxicodynamic (TD) model was evaluated to characterize the PK of CMS/CMS-E2 and colistin/colistin B in plasma as well as its relationship with nephrotoxicity. Creatinine (CR) and blood urea nitrogen (BUN) profiles were described using an indirect link PK-TD model, with linear-effect relationship. Both the slope between colistin or colistin B concentrations in the effect compartment and CR, BUN was significantly lower for CMS-E2 compared with CMS (CR: $P = 0.027$, BUN: $P = 0.043$). The concentrations of colistin and colistin B in kidneys were correlated with CR, BUN values, and histologic examination scores. The regression coefficient of CMS-E2 between the colistin B concentrations in renal tissues and CR, BUN values were lower, as well (CR: $P = 0.003$, BUN: $P = 0.001$). The renal injuries induced by CMS and CMS-E2 lagged behind the change of plasma colistin or colistin B concentrations and correlated to those in kidneys. CMS-E2 showed significantly lower nephrotoxicity compared to CMS in vivo.

Keywords Colistin, Colistin methanesulphonate (CMS), Colistin B, Polymyxins, Pharmacokinetic-toxicodynamic, Nephrotoxicity

Colistin is a class of cationic antimicrobial cyclic lipopeptides, which kills bacteria by disturbing cell outer membranes[1]. Its prodrug, colistin methanesulphonate (CMS), which can transform to colistin in vivo, is used in clinics. Although CMS was introduced in the late 1950s, its use declined in the 1970s due to potential nephrotoxicity and neurotoxicity[2]. However, since the 2000s, the increasing prevalence of multi-drug antibiotic resistance has led to a resurgence in the use of colistin. It has become a last resort for treating infections caused by carbapenem-resistant *Pseudomonas aeruginosa*, *Pseudomonas baumannii*, and *Klebsiella pneumoniae*[3–7].

Nevertheless, the high incidence of nephrotoxicity has limited its clinical application[8]. Meta-analyses have shown that the incidence of CMS-associated nephrotoxicity can reach up to 48%[9]. Several studies have demonstrated that CMS nephrotoxicity is closely related to plasma colistin concentrations. Sorli et al.[10] showed that a steady-state trough concentration of > 2.2 mg/L was associated with increased nephrotoxicity. Similarly, Forrest et al.[11] demonstrated that colistin steady-state average concentrations of > 1.88 mg/L and > 2.25 mg/L

[1]Institute of Antibiotics, Huashan Hospital, Fudan University, Shanghai 200040, China. [2]Key Laboratory of Clinical Pharmacology of Antibiotics, Shanghai 200040, China. [3]National Clinical Research Center for Aging and Medicine, Huashan Hospital, Fudan University, Shanghai 200040, China. [4]Clinical Pharmacology Research Center, Huashan Hospital, Fudan University, Shanghai 200040, China. [5]Chenxue Guo and Lin Xi contributed equally to this work. ✉email: xiaofenliu@fudan.edu.cn; zhangj61@fudan.edu.cn

increased the incidence of nephrotoxicity in patients with baseline creatinine (CR) clearance < 80 mL/min and ≥ 80 mL/min, respectively. Therefore, understanding the pharmacokinetics (PK)–nephrotoxicity relationship of CMS could help optimize dosing to reduce toxicity. Although the accumulation of polymyxins in the kidneys[12,13] is thought to be associated with nephrotoxicity, obtaining clinical data is challenging. Thus, we also explored the PK–nephrotoxicity relationships of CMS from the perspective of kidney drug concentrations in rats.

To date, 36 different components have been identified in colistin[14], with colistin A and B being the main components[15]. The only difference between these two main components is the presence of an additional methylene group at the N-terminal fatty acyl group and a chiral center at the 6th position of the fatty acyl chain[16]. One study revealed that, at the identical dose of 0.75 mM, the apoptotic effect of colistin A was three times that of colistin B on human kidney proximal tubular HK-2 cells, although there was no difference in antibacterial activity between the two components in vitro and in vivo[17]. Therefore, colistin B methanesulphonate (CMS-E2) has been developed with an expectation of lower toxicity. It is essential to quantify its nephrotoxicity in vivo, compare it with CMS, and clarify its relationship with PK. It is also worth noting the likely difference in PK between CMS and CMS-E2. One study found that the clearance of colistin B was almost half that of colistin[16]. Additionally, there is variability in the PK of methanesulphonate derivatives. The PK of CMS has been reported to vary between brands[18]. Given the complexity of the composition of CMS as methanesulphonate derivatives[19,20], the process of CMS converting to colistin in vivo could be variable. In general, the PK of CMS-E2 and the formed colistin B could differ from that of CMS. Considering these potential differences in PK, elucidating the PK-nephrotoxicity relationships of CMS and CMS-E2 could also help compare the renal toxicity of the two drugs.

This study described the PK of CMS, CMS-E2, and their active forms in plasma and kidneys, as well as the changes in nephrotoxicity induced by the two drugs over time. The relationships between drug exposure and injury severity in plasma and kidneys were established. The aim of this study is to gain a better understanding of the PK-nephrotoxicity relationships of CMS and CMS-E2 and to conduct a preliminary comparison of their toxicity, in order to gain a greater benefit between therapeutic outcomes and nephrotoxicity in future clinical applications of these drugs.

Results

PK of CMS and CMS-E2 and formed colistin/colistin B in plasma

The maximal tolerability dose of CMS-E2 [40 mg/kg colistin base activity (CBA)] was significantly higher than that of CMS (20 mg/kg CBA) in rats, and both maximal and half-maximal tolerability doses were selected as dose regimens. Figure 1 illustrates the mean (± standard deviations, SD) plasma concentrations-time profiles of CMS/CMS-E2 and the formed colistin/colistin B following intraperitoneal administration of CMS and CMS-E2. The PK parameters of the non-compartmental analysis (NCA) model are presented in Table 1. Significant differences in terminal half-life ($t_{1/2}$) and total body clearance/bioavailability (CL/F) were observed between dosage groups. The $t_{1/2}$ of CMS administrated at 20 mg/kg CBA (76.4 ± 33.4 min) was significantly longer than that at 10 mg/kg CBA (27.2 ± 4.08 min, $P = 0.024$), a trend also observed for colistin (113 ± 40.3 min vs. 44.4 ± 4.61 min, $P = 0.017$). A similar pattern was seen for the $t_{1/2}$ of CMS-E2 (20 mg/kg, 34.4 ± 9.62 min vs. 40 mg/kg, 94.0 ± 31.3 min, $P = 0.030$), while the $t_{1/2}$ of colistin B did not show a statistically significant difference between dose groups (20 mg/kg, 73.1 ± 18.8 min vs. 40 mg/kg, 128 ± 49.6 min, $P = 0.157$). The CL/F of CMS after a dose of 20 mg/kg CBA (2.99 ± 2.02 mL/min/kg`) was significantly lower than that after 10 mg/kg CBA (5.42 ± 0.132 mL/min/kg, $P = 0.043$). Similarly, the CL/F of CMS-E2 after a dose of 40 mg/kg CBA (4.18 ± 2.56 mL/min/kg) was marginally lower compared to that after 20 mg/kg CBA (9.90 ± 3.34 mL/min/kg, $P = 0.053$).

We compared the PK of CMS and CMS-E2 at the same dose (20 mg/kg CBA). The maximum observed concentration (C_{max}) and area under the concentration-time curve to infinite time (AUC_{0-inf}) of both the prodrug and active form of CMS were significantly higher than those of CMS-E2 (C_{max}: CMS, 39.5 ± 13.7 mg/L vs. CMS-E2, 19.1 ± 4.50 mg/L, $P = 0.037$; colistin, 3.99 ± 1.52 mg/L vs. colistin B, 1.14 ± 0.217 mg/L, $P = 0.011$; AUC_{0-inf}: CMS, 149 ± 69.6 mg·h/L vs. CMS-E2, 37.2 ± 10.5 mg·h/L, $P = 0.025$; AUC_{0-inf}: colistin, 23.1 ± 7.46 mg·h/L vs. colistin B, 4.14 ± 1.26 mg·h/L, $P = 0.005$). The $t_{1/2}$ of CMS and colistin were longer compared to those of CMS-E2 and colistin B, although the difference for the active form was not statistically significant (prodrug, $P = 0.036$; active form, $P = 0.185$). The CL/F and the volume of distribution/bioavailability (V_d/F) of CMS were lower (CL/F: CMS, 2.99 ± 2.02 mL/min/kg vs. CMS-E2, 9.90 ± 3.34 mL/min/kg, $P = 0.024$; V_d/F: CMS, 266 ± 81.6 mL/kg vs. CMS-E2, 464 ± 108 mL/kg, $P = 0.021$).

PK of CMS/CMS-E2 and formed colistin/colistin B in kidneys

Figure 2 illustrates the kidney concentration-time profiles of CMS/CMS-E2 and the formed colistin/colistin B, with corresponding PK parameters presented in Table 2. Both prodrugs and their active forms accumulated significantly in the kidneys, particularly colistin and colistin B, which exhibited prolonged residence times. Besides, the time of maximum observed concentration (T_{max}) in renal tissues occurred later than that in plasma. The maximum kidney concentrations of CMS and colistin administrated at 10 mg/kg CBA were 64.2 ± 38.1 μg/g and 10.4 ± 3.13 μg/g at 2 h, respectively, which were 3.0 and 13.7 times higher than those in plasma. A relatively high colistin concentration (3.55 ± 0.594 μg/g) was still present at 8 h. After a dose of 20 mg/kg CBA of CMS, the C_{max} of CMS (281 ± 92.8 μg/g at 4 h) and colistin (53.6 ± 11.0 μg/g at 6 h) in the kidneys were 6.5 and 12.7 times higher than those in plasma, respectively. The colistin concentration remained at 5.97 ± 1.85 μg/g at 24 h. The maximum kidney concentrations of CMS-E2 and colistin B administrated at 20 mg/kg CBA were 105 ± 25.3 μg/g (at 2 h) and 17.6 ± 6.79 μg/g (at 4 h), respectively, which were 5.5 and 12.7 times higher than those in plasma. The colistin B concentration was 4.86 ± 2.36 μg/g at 8 h. After a dose of 40 mg/kg CBA of CMS-E2, the C_{max} of CMS-E2 (379 ± 85.5 μg/g at 4 h) and colistin B (148 ± 32.9 μg/g at 10 h) in the kidneys were 7.6 and 20.1 higher than those in plasma, respectively. A relatively high colistin B concentration (11.5 ± 1.74 μg/g) was still observed at 24 h.

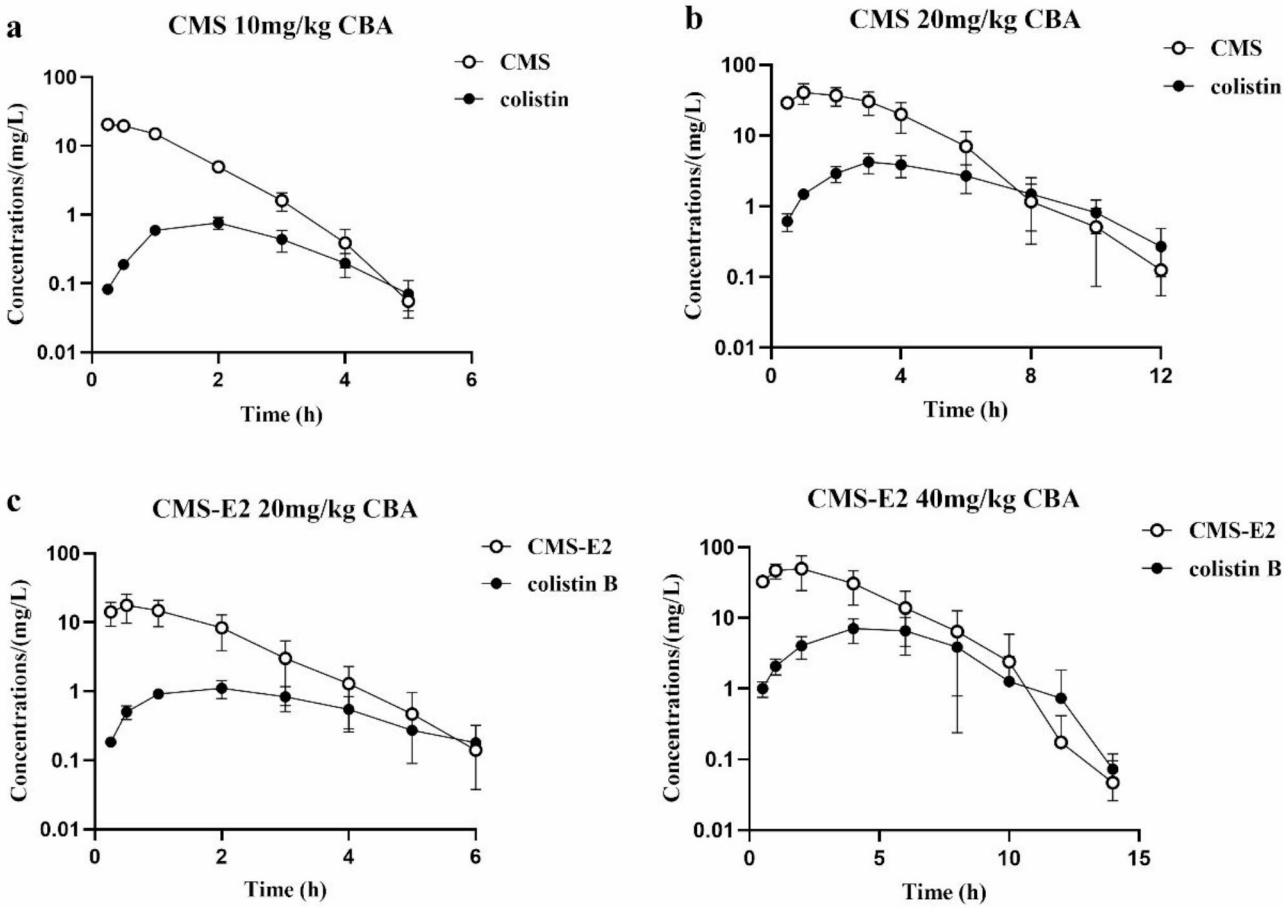

Fig. 1. Mean (±SD) plasma concentrations of CMS/CMS-E2 and colistin/colistin B versus time following intraperitoneal administration of CMS at 10, 20 mg/kg CBA and CMS-E2 at 20, 40 mg/kg CBA. *CMS* colistin methanesulphonate, *CMS-E2* colistin B methanesulphonate, *CBA* colistin base activity.

Acute kidney injury evaluation

Figure 3A and C display the mean (±SD) plasma CR and blood urea nitrogen (BUN) values over time after administration. Following the administration of CMS at 10 and 20 mg/kg CBA, peak CR levels were observed at 2.67 ± 1.70 h and 5.36 ± 1.30 h, respectively. The peak time for BUN was 5.00 ± 0.00 h and 7.82 ± 1.34 h. When 20 and 40 mg/kg CBA of CMS-E2 were administered, CR peaks were reached at 4.67 ± 1.89 h and 8.86 ± 3.18 h; while BUN peaked at 6.00 ± 0.00 h and 11.1 ± 3.00 h, respectively. Notably, there was a lag time between the plasma maximal colistin or colistin B concentrations and the peak CR and BUN levels. The peak CR and BUN levels increased with the increased dose of CMS and CMS-E2 (CR: CMS, 31.3 ± 5.41 μmol/L vs. 93.1 ± 29.8 μmol/L, $P < 0.001$, CMS-E2, 33.4 ± 3.44 μmol/L vs. 104 ± 40.4 μmol/L, $P < 0.001$, Fig. 3B; BUN: CMS, 8.09 ± 1.66 mmol/L vs. 17.8 ± 3.83 mmol/L, $P < 0.001$, CMS-E2, 12.4 ± 5.21 mmol/L vs. 23.0 ± 5.59 mmol/L, $P = 0.043$, Fig. 3D). We also compared the peak levels of biomarkers at the same dose (20 mg/kg CBA). The elevation of CR in the CMS groups was more significant than that in the CMS-E2 groups ($P < 0.001$, Fig. 3B), and the difference in BUN between the two drugs was marginally significant ($P = 0.067$, Fig. 3D).

Figure 3E shows the changes in the semi-quantitative score (SQS) over time. Representative photomicrographs of each group at each time point are shown in Figure S1-4. No obvious histological lesion were observed during the first 2 h after administration of CMS at 10, 20 mg/kg CBA and CMS-E2 at 20 mg/kg CBA. The histological damage was most severe at 6 h after administration of CMS at 10 mg/kg CBA, with an SQS score of + 2 when plasma concentrations fell below the limit of detection and most of the damage was completely recovered at 8 h. For the rats administered 20 mg/kg CBA of CMS-E2, only two of the three rats had transient mild lesions with an SQS score of + 1 at 4 h, and no obvious damage was observed at other time points. The most severe histological abnormalities were observed at 6 to 12 h after administration of CMS at 20 mg/kg CBA, with an SQS score of + 3, and at 4 to 14 h after administration of CMS-E2 at 40 mg/kg CBA, with an SQS score of + 2, and all damage was completely recovered at 24 h. Obviously, the duration of kidney tissue damage was longer in the higher-dose groups. Otherwise, the severity of damage increased with the elevation of the dose (CMS, 2.00 ± 0.00 vs. 3.00 ± 0.00, $P = 0.025$; CMS-E2, 0.667 ± 0.577 vs. 2.33 ± 1.16, $P = 0.043$, Fig. 3F). We also compared the maximal SQS at the same dose (20 mg/kg CBA), which was significantly higher than in the CMS groups than in the CMS-E2 groups ($P = 0.034$, Fig. 3F).

Drug	Dosage (mg·kg CBA)	T_{max} (h)		C_{max} (mg/L)		$t_{1/2}$ (min)		CL/F (mL/min/kg)	V_d/F (mL/kg)	AUC_{0-inf} (mg·h/L)		$AUC_{0-inf,\ colistin}$/ $AUC_{0-inf,\ CMS}$ or $AUC_{0-inf,\ colistin\ B}$/ $AUC_{0-inf,\ CMS-E2}$
		CMS/CMS-E2	colistin/ colistin B	CMS/CMS-E2	colistin/ colistin B	CMS/CMS-E2	colistin/ colistin B	CMS/CMS-E2	CMS/CMS-E2	CMS/CMS-E2	colistin/ colistin B	
CMS	10	0.333 ± 0.118	2.00 ± 0.00	21.4 ± 0.727	0.764 ± 0.123	27.2 ± 4.08	44.4 ± 4.61	5.42 ± 0.132	213 ± 36.2	30.7 ± 0.763	2.05 ± 0.371	0.0665 ± 0.0110
	20	1.30 ± 0.458	3.55 ± 0.891	39.5 ± 13.7	3.99 ± 1.52	76.4 ± 33.4	113 ± 40.3	2.99 ± 2.02	266 ± 81.6	149 ± 69.6	23.1 ± 7.46	0.179 ± 0.0368
CMS-E2	20	0.500 ± 0.00	1.67 ± 0.471	19.1 ± 4.50	1.14 ± 0.217	34.4 ± 9.62	73.1 ± 18.8	9.90 ± 3.34	464 ± 108	37.2 ± 10.5	4.14 ± 1.26	0.111 ± 0.00664
	40	1.75 ± 0.433	4.71 ± 0.881	50.9 ± 23.5	7.08 ± 2.77	94.0 ± 31.3	128 ± 49.6	4.18 ± 2.56	497 ± 278	226 ± 125	35.5 ± 23.9	0.222 ± 0.0323

Table 1. NCA PK parameters estimates in plasma of CMS/CMS-E2 and formed colistin/colistin B in rats. *CMS* colistin methanesulphonate, *CMS-E2* colistin B methanesulphonate, *CBA* colistin base activity, T_{max} time of maximum observed concentration, C_{max} maximum observed concentration, $t_{1/2}$ terminal half-life, *CL/F* total body clearance/bioavailability, *Vd/F* volume of distribution/ bioavailability, AUC_{0-inf} area under the concentration-time curve to infinite time.

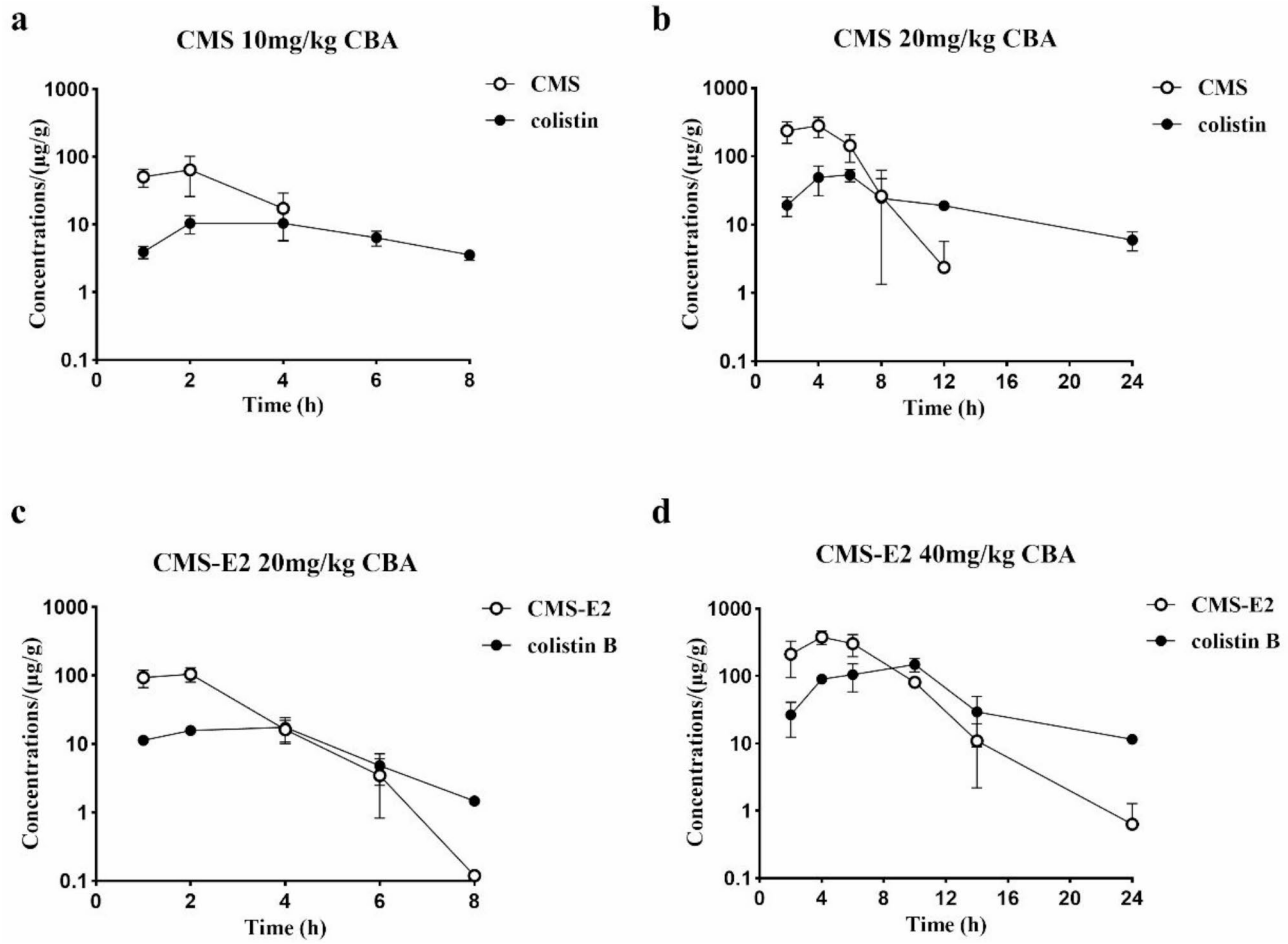

Fig. 2. Mean (± SD) concentrations of CMS/CMS-E2 and colistin/colistin B in renal tissue versus time following intraperitoneal administration of CMS at 10, 20 mg/kg CBA and CMS-E2 at 20, 40 mg/kg CBA. (The CMS concentration was below the lower limit 6, 8 h after administration of CMS 10 mg/kg CBA and 24 h after administration of CMS 20 mg/kg CBA). *CMS* colistin methanesulphonate, *CMS-E2* colistin B methanesulphonate, *CBA* colistin base activity.

PK-toxicodynamic (TD) modeling of plasma PK and biomarkers for CMS and CMS-E2

We further developed a PK-TD model to elucidate the abovementioned lag time and the relationships between plasma drug concentrations and CR and BUN levels. The schematic representation of the PK-TD model is shown in Fig. 4A. The plasma CMS/CMS-E2 and formed colistin/colistin B concentrations, as well as the values of CR and BUN, were well predicted by the model (Figure S5). In the PK component, there's one compartmental model to fit the kinetics of drug concentrations of CMS/CMS-E2 and formed colistin/colistin B. $K_{CMS,\ colistin}$/$K_{CMS-E2,\ colistin\ B}$ represents the rate constant for CMS/CMS-E2 conversion into colistin/colistin B. Elimination from both the CMS/CMS-E2 and the colistin/colistin B central plasma compartments is described by a linear first-order total clearance. In the TD component, we incorporated an indirect hypothetic effect compartment to simulate the delay in nephrotoxicity. K_e represents the elimination rate constant from the central compartment of colistin/colistin B to the effect compartment. To facilitate a clearer understanding, we calculated the time lag (T_{lag}) by taking the reciprocal of K_e, which signifies the temporal delay between the toxic effects and PK changes. The corresponding parameters are presented in Table 3. The clearance of CMS (CL_{CMS}) or CMS-E2 (CL_{CMS-E2}) of the high-dose group was significantly lower than that in the low-dose group (CL_{CMS}: 2.52 ± 1.64 mL/min/kg vs. 5.14 ± 0.196 mL/min/kg, $P = 0.035$; CL_{CMS-E2}: 3.2 ± 1.74 mL/min/kg vs. 9.69 ± 3.95 mL/min/kg, $P = 0.016$). The clearance of colistin ($CL_{colistin}$) or colistin B ($CL_{colistin\ B}$) showed the same trend, and the difference was not statistically significant ($CL_{colistin}$: 20 mg/kg, 1.75 ± 1.21 mL/min/kg vs. 10 mg/kg, 2.57 ± 0.420 mL/min/kg, $P = 0.139$; $CL_{colistin\ B}$: 40 mg/kg, 1.89 ± 0.809 mL/min/kg vs. 20 mg/kg, 3.31 ± 1.03 mL/min/kg, $P = 0.087$). Peak CR levels were used to explore the relationship between renal function and drug clearance in the present study. The results indicated that decreased drug clearance (including the prodrugs and their formed activity components of CMS and CMS-E2) was associated with elevated CR levels (CL_{CMS}, $P < 0.001$, CL_{CMS-E2}, $P = 0.011$, $CL_{colistin}$, $P = 0.020$, $CL_{colistin\ B}$, $P = 0.003$, Fig. 4B). According to the PK-TD model for CMS and CMS-E2, the T_{lag} for CR were 1.73 ± 1.22 h and 1.83 ± 1.05 h, respectively, while those for BUN were 8.45 ± 4.08 h and 11.9 ± 9.46 h, respectively. Moreover, linear relationships were observed between CR, BUN values, and colistin/colistin B

Drug	Dosage (mg·kg CBA)	T_{max} (h)		C_{max} (μg/g)		Kidney/plasma ratio of C_{max}		AUC_{0-inf} or AUC_{last} (μg·h/g)		Kidney/plasma ratio of AUC_{0-inf}		$t_{1/2}$ (min)	
		CMS/CMS-E2	Colistin/colistin B	CMS/CMS-E2	Colistin/colistin B	CMS/CMS-E2	Colistin/colistin B	CMS/CMS-E2	Colistin/colistin B	CMS/CMS-E2	Colistin/colistin B	CMS/CMS-E2	Colistin/colistin B
CMS	10	2	2	64.2 ± 38.1	10.4 ± 3.13	3.00	13.7	162*	64.7	5.24	31.6	–	72.6
	20	4	6	281 ± 92.8	53.6 ± 11.0	7.11	12.7	1620	603	10.9	26.1	61.8	374
CMS-E2	20	2	4	105 ± 25.3	17.6 ± 6.79	5.50	15.4	289	81.5*	7.80	19.7	33.6	–
	40	4	10	379 ± 85.5	148 ± 32.8	7.44	20.1	2496	1406*	11.0	39.6	121	–

Table 2. NCA PK parameters estimates in kidneys of CMS/CMS-E2 and formed colistin/colistin B in rats. *The value was AUC_{last}. *CMS* colistin methanesulphonate, *CMS-E2* colistin B methanesulphonate, *CBA* colistin base activity, T_{max} time of maximum observed concentration, C_{max} maximum observed concentration, AUC_{0-inf} area under the concentration-time curve to infinite time, AUC_{last} area under the curve from time 0 to last measurable concentration, $t_{1/2}$ terminal half-life.

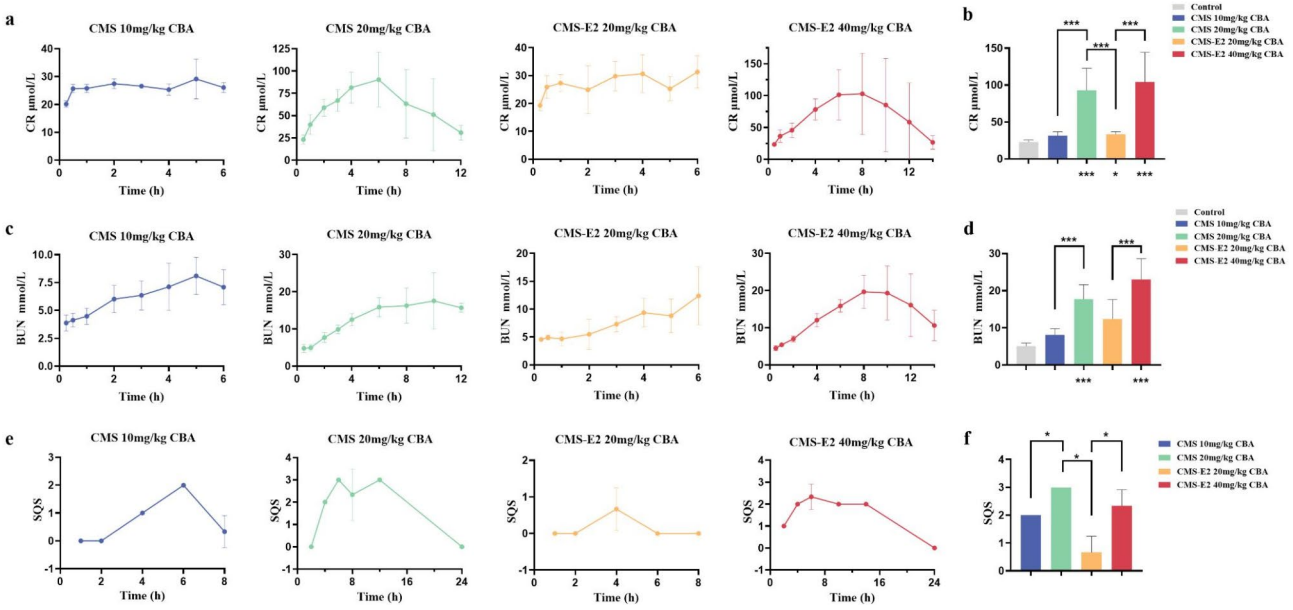

Fig. 3. Changes of acute kidney injuries over time and comparison between groups. Mean (± SD) plasma values of (A) CR, (C) BUN and (E) SQS versus time following intraperitoneal administration of CMS at 10, 20 mg/kg CBA and CMS-E2 at 20, 40 mg/kg CBA. The peak level of (B) CR, (D) BUN and (F) SQS among control and all dose groups. * below each bar represents the difference between this group and the control group in (B) and (D). Because the SQS in the control group was 0, it was not displayed in (F). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. *CMS* colistin methanesulphonate, *CMS-E2* colistin B methanesulphonate, *CBA* colistin base activity, *CR*, creatinine, *BUN* blood urea nitrogen, *SQS* semiquantitative score.

concentrations in the effect compartment derived from the PK-TD model. For both CR and BUN, the predicted slope of the linear equation between colistin B concentrations in the effect compartment and the effect was significantly lower for CMS-E2 than CMS (CR: 13.0 ± 3.52 mmol/g vs. 20.9 ± 9.13 mmol/g, $P = 0.027$; BUN: 7.78 ± 4.56 mol/g vs. 11.7 ± 7.27 mol/g, $P = 0.043$).

Relationship between kidney PK and injury of CMS and CMS-E2

We further investigated the relationships between drug concentrations in the kidneys and renal injuries. The results suggested that the concentrations of colistin or colistin B in the kidneys were linearly correlated with contemporaneous CR [colistin: 0.74, 95% confidence interval (CI), 0.50–0.97, $P < 0.001$, Fig. 5A; colistin B: 1.58, 95% CI, 1.25–1.90, $P < 0.001$, Fig. 5D], BUN values (colistin: 0.27, 95% CI, 0.20–0.35, $P < 0.001$, Fig. 5B; colistin B: 0.08, 95% CI, 0.04–0.12, $P < 0.001$, Fig. 5E), and SQS (colistin: 0.10, 95% CI, 0.04–0.16, $P = 0.001$, Fig. 5C; colistin B: 0.03, 95% CI, 0.01–0.06, $P = 0.011$, Fig. 5F), but not with the concentrations of CMS or CMS-E2 (Figure S6). We then compared the regression coefficient of the two drugs. For both CR and BUN, the regression coefficient between colistin B concentrations in renal tissues and the effect was significantly lower for CMS-E2 than CMS (CR, $P = 0.003$; BUN, $P = 0.001$).

Discussion

Nephrotoxicity has been a major limiting factor for CMS clinical application[8]. CMS-E2 was developed based on the consideration that the single component colistin B exhibits lower nephrotoxicity compared with traditional colistin. Consequently, we investigated the PK-nephrotoxicity relationships of CMS and CMS-E2 by analyzing plasma and kidney drug concentrations, while comparing the toxicity profiles of the two drugs in rats.

Previous studies have investigated the PK of CMS in rats. Considering the differences in administration routes, we focused only on the $t_{1/2}$ of drugs. Prior reports indicate a $t_{1/2}$ range of 20 to 40 min for CMS, and 32–108 min for formed colistin[18,21,22]. Consistent with these findings, our study observed $t_{1/2}$ values of 27.2 ± 4.08 min for CMS and 76.4 ± 33.4 min for formed colistin in the low-dose group. Notably, significant PK differences emerged between the two dose groups of CMS and CMS-E2. Both CL_{CMS}/CL_{CMS-E2} and $CL_{colistin}/CL_{colistin\ B}$ showed significantly decreased clearance in high-dose groups. This decreased drug clearance correlated with kidney injury, a phenomenon also observed clinically[23]. As CMS is primarily excreted via glomerular filtration and tubular secretion[22,24,25], its clearance naturally declines with impaired renal function. In contrast, colistin elimination occurs mainly though non-renal pathways[26,27], with only 18% undergoing glomerular filtration into the tubular lumen, most of which then is reabsorbed through the epithelial tubular cells[28]. Previous studies have shown that reabsorbed colistin appears to undergo intracellular metabolism rather than re-entering systemic circulation[28,29]. The decline of colistin and colistin B clearance is possibly associated with impaired glomerular filtration. This may also explain why renal function demonstrates weaker effects on their clearance compared to CMS/CMS-E2.

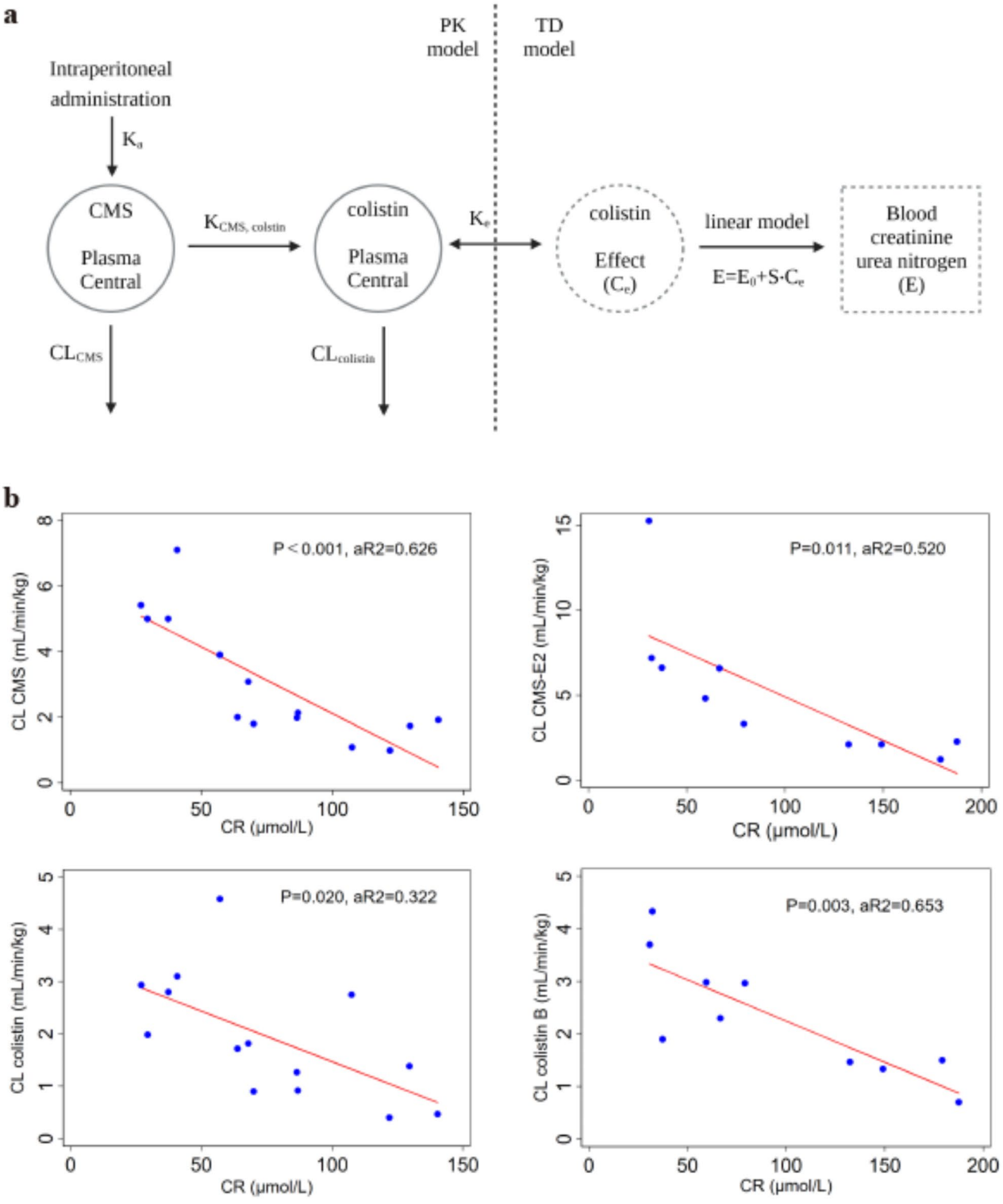

Fig. 4. PK/TD modeling of plasma PK and biomarkers of CMS and CMS-E2. A, Schematic representation of a one-compartmental PK model in which the hypothetical effect compartment is indirectly linked to the plasma pharmacokinetic compartment (take CMS for example). B, Relationship between CL_{CMS}, CL_{CMS-E2}, $CL_{colistin,}$ and $CL_{colistin\ B}$ and the value of CR. CMS, colistin methanesulphonate; CMS-E2, colistin B methanesulphonate; CL_{CMS}, clearance of CMS; CL_{CMS-E2}, clearance of CMS-E2; $CL_{colistin}$, clearance of colistin; $CL_{colistin\ B}$, clearance of colistin B; K_a, absorption rate; $K_{CMS, colistin}$, the rate constant for CMS conversion into colistin; K_e, elimination rate from colistin central compartment to effect compartment; E, level of plasma creatinine and urea nitrogen in CMS and CMS-E2-induced nephrotoxicity; E_0, baseline level of plasma creatinine and urea nitrogen prior to drug administration; S, proportional coefficient between drug concentrations and the effect; C_e, colistin concentrations in effect compartment, *CR* creatinine.

Drug	PK					
	Dose (mg·kg CBA)	$K_{a\ CMS/CMS-E2}$(1/h)	$CL_{CMS/CMS-E2}$ (mL/min/kg)	$CL_{colistin/colistin\ B}$ (mL/min/kg)	V (mL/kg)	$K_{CMS,\ colistin}/K_{CMS-E2,\ colistin\ B}$ (mL/min/kg)
CMS	10	2.02 ± 0.260	5.14 ± 0.196	2.57 ± 0.420	194 ± 12.8	0.756 ± 0.0208
	20	0.878 ± 0.342	2.52 ± 1.64	1.75 ± 1.21	199 ± 84.6	1.21 ± 0.381
CMS-E2	20	1.20 ± 0.253	9.69 ± 3.95	3.31 ± 1.03	263 ± 58.3	1.40 ± 0.125
	40	0.673 ± 0.208	3.20 ± 1.74	1.89 ± 0.809	291 ± 105	1.46 ± 0.124
	TD					
	Drug	K_e (1/h)	T_{lag} (h)	E_0 (CR: μmol/L, BUN: mmol/L)	S (CR: mmol/g, BUN: mol/g)	
CR	CMS	0.833 ± 0.555	1.73 ± 1.22	24.2 ± 3.65	20.9 ± 9.13	
	CMS-E2	0.814 ± 0.524	1.83 ± 1.05	23.0 ± 4.63	13.0 ± 3.52	
BUN	CMS	0.157 ± 0.100	8.45 ± 4.08	4.54 ± 0.841	11.7 ± 7.27	
	CMS-E2	0.182 ± 0.164	11.9 ± 9.46	4.01 ± 0.789	7.78 ± 4.56	

Table 3. PK/TD parameters estimates following intraperitoneal administration of CMS and CMS-E2. *CMS* colistin methanesulphonate, *CMS-E2* colistin B methanesulphonate, *CBA* colistin base activity, $K_{a\ CMS/CMS-E2}$, absorption rate of CMS/CMS-E2; $CL_{CMS/CLCMS-E2}$, total body clearance of CMS/CMS-E2; $CL_{colistin/CLcolistin\ B}$, total body clearance of colistin/colistin B; V, volume of distribution; $K_{CMS,\ colistin}/K_{CMS-E2,\ colistin\ B}$, metabolic rate from CMS/CMS-E2 compartment to colistin/colistin B compartment; K_e, elimination rate from colistin/colistin B central compartment to effect compartment; T_{lag}, lag time; E_0, baseline level of blood creatinine and urea nitrogen prior to drug administration; S, proportional coefficient between the drug concentrations and the effect; *CR* creatinine, *BUN* blood urea nitrogen.

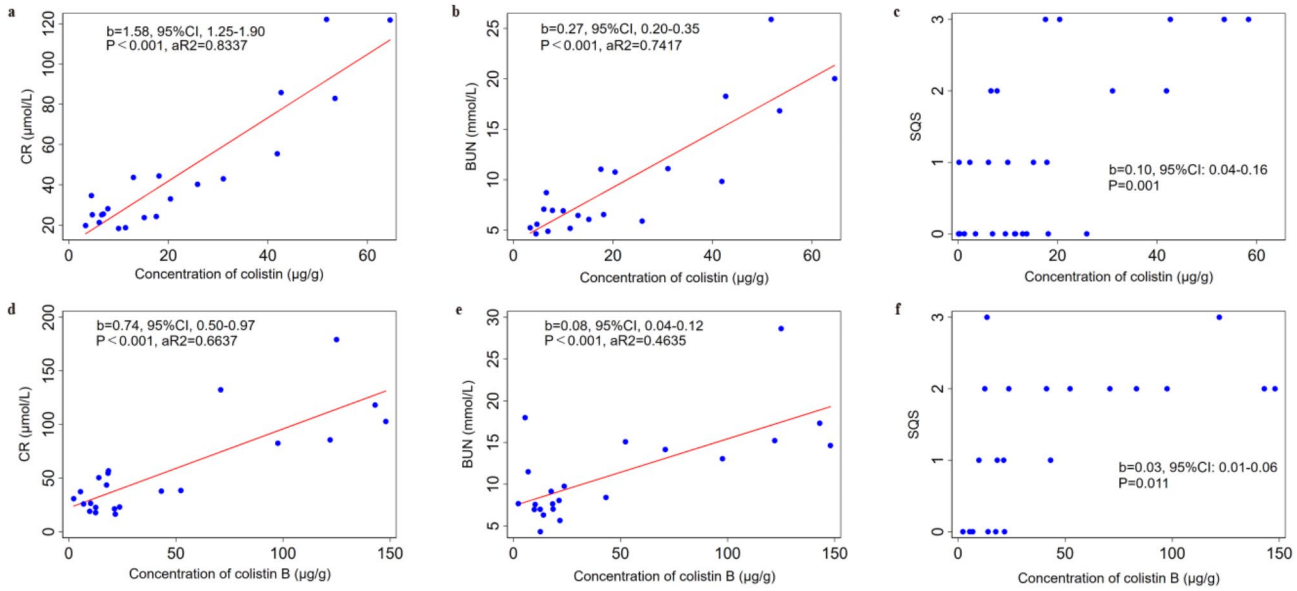

Fig. 5. Relationship between the renal tissue concentrations of colistin (upper) and colistin B (bottom) and the values of CR, BUN, and SQS (from left to right). *CR* creatinine, *BUN* blood urea nitrogen, *SQS* semiquantitative score.

Our study provides the first PK characterization of CMS-E2 in rats relative to CMS. It should be noted that CMS PK exhibits brand-to-brand variability due to component complexity[18]. The methanesulphonation process generates diverse CMS molecules with varying numbers and positions of methanesulphonate groups[19,20], making CMS a heterogeneous mixture. To ensure comparability, CMS and CMS-E2 in this study were manufactured using identical processes. Notwithstanding this, significant PK differences emerged: At equivalent CBA doses (20 mg/kg), CMS showed higher drug exposure than CMS-E2, suggesting CMS-E2 may require dose escalation to achieve comparable plasma concentrations. Furthermore, CMS-E2 displayed significantly larger V_d/F. This observation aligns with Sivashangarie et al.'s findings[16] that colistin B exhibits a higher V_d/F than colistin A, potentially reflecting differential plasma protein binding (41.7 ± 12.4% vs. 56.6 ± 9.25%)[16], which is likely due to structure difference between the two compounds. The $t_{1/2}$ and the CL/F were significantly different between CMS and CMS-E2 in the present study. Supporting this, Sivashangarie et al.[16] reported colistin B clearance of

3.05 ± 0.62 mL/min/kg when administered as a single component, lower than values observed for that (5.2 ± 0.4 mL/min/kg) after the colistin sulfate[27]. These findings underscore the importance of conducting additional PK studies on CMS-E2 given methanesulphonate derivative variability.

Both prodrugs and active forms showed significant kidney accumulation. While prior studies quantified renal accumulation using simultaneous tissue-to-plasma concentration ratios[12,13,28], our data revealed asynchronous PK between kidneys and plasma, with delayed T_{max} and prolonged residence time in renal tissue. The renal/plasma C_{max} ratio reached 3.00-7.44 for CMS/CMS-E2, consistent with that when CMS reached a steady state after administration[30]. Similarly, formed colistin/colistin B showed 12.7–20.1× ratios, matching post-colistin sulfate administration observations[30]. This implied that CMS did not increase the colistin concentration in kidneys. A physiologically-based PK model confirms renal colistin originates primarily from plasma[29], with minimal (< 1%) renal CMS conversion[29]. Notably, like polymyxin B[12,13], detectable colistin/colistin B levels persisted in kidneys long after plasma concentrations became undetectable.

Biochemical and histological analyses revealed dose-dependent nephrotoxicity for both CMS and CMS-E2 in rats, mirroring clinical CMS observations[31–35]. Importantly, mild renal dysfunction (CR/BUN elevation) induced by these agents represented functional disturbances rather than structural damage.

We developed a PK-TD model to elucidate plasma concentration-injury biomarker relationships. The model demonstrated linear CR/BUN correlations with effect compartment colistin/colistin B concentrations within limited ranges. Moreover, CR, BUN, and SQS correlated with renal tissue concentrations, explaining injury "lag time" and prolonged persistence. This finding could also help to provide new insights into some debates related to CMS- and CMS-E2-induced nephrotoxicity: At low renal concentrations, effects manifest as reversible functional impairment, while higher concentrations promote irreversible pathological damage. Thus, minimizing peak-trough fluctuations with a lower renal C_{max} could mitigate injury at equivalent cumulative exposures. More than that, contrary to suggestions that a higher renal C_{max} limits renal accumulation due to tubular reabsorption saturation[36], our data showed unchanged renal/plasma ratios with increasing doses, indicating no saturation at conventional rat doses. Collectively, more frequent dosing appears less nephrotoxic. This hypothesis aligns with reports of reduced nephrotoxicity with thrice-daily vs. twice-daily dosing in rats[37].

Comparative toxicity of CMS and polymyxin B is an important question in the selection of clinical applications of polymyxins. Meta-analysis demonstrated that the incidence of nephrotoxicity was higher with CMS than with polymyxin B[9]. However, the inconsistent dosage of the two drugs makes the results controversial[38]. Studies reported that colistin was slightly less toxic to tubular cells than polymyxin B in vitro[17,39]. The possible less toxicity of polymyxin B was thought to be related to the larger peak-to-trough fluctuation compared with CMS[40]. Nevertheless, as mentioned above, a smaller concentration fluctuation tends to be less nephrotoxic. Besides, the conversion of CMS in kidneys was thought to increase the concentration of colistin, which could further increase renal toxicity[40]. Our findings, however, showed the opposite that CMS did not cause higher renal colistin concentrations. Whether CMS itself increases renal toxicity remains unclear. CMS also accumulates in large quantities in kidneys, although it is less toxic than colistin[19]. More preclinical and clinical studies are still needed to compare the nephrotoxicity between CMS and polymyxin B, especially at an effective concentration.

To our knowledge, this constitutes the first in vivo comparison of CMS and CMS-E2 nephrotoxicity. At equivalent CBA doses (20 mg/kg), CMS produced greater biochemical/histological damage than CMS-E2. However, given their distinct PK profiles, we employed concentration-effect coefficients in the PK-nephrotoxicity modeling to compare the nephrotoxicity. Our data further revealed that both the slope between colistin or colistin B concentrations in the effect compartment and CR and BUN were significantly lower for CMS-E2 compared with CMS. Similarly, CMS-E2 showed lower regression coefficients between renal colistin B concentrations and CR/BUN. These findings align with in vitro evidence that colistin B induces less HK-2 cell apoptosis than colistin A or colistin[17]. Our results conclusively demonstrate CMS-E2's superior renal safety profile in rats.

Conclusions

This study elucidated the pharmacokinetic-nephrotoxicity relationships of CMS and CMS-E2 through comprehensive analysis of plasma and renal drug concentrations, while systematically comparing their nephrotoxic profiles in rats. The time-delayed indirect-link PK-TD model proved effective in characterizing the association between plasma PK and nephrotoxicity for both agents. Notably, drug-induced nephrotoxicity correlated strongly with renal tissue concentrations of colistin/colistin B. Critically, CMS-E2 exhibited significantly lower nephrotoxicity compared to CMS. These findings not only provide novel insights into CMS-associated renal toxicity but also propose CMS-E2 as a safer alternative for optimizing polymyxin-based therapies in clinical practice.

Materials and methods

Animals

Male Sprague-Dawley rats (body weight, 300 ± 15 g) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. The present study was approved by the Animal Welfare and Ethics Group of the Department of Laboratory Animal Science, Fudan University (Approval No. 202110012 S). All experimental procedures were conducted in compliance with ARRIVE guidelines and relevant regulations. Animals were housed under controlled conditions (22 °C, 50% relative humidity, 12-hour light/dark cycle) with ad libitum access to food and water. After a 1-week acclimatization period, rats were euthanized via exsanguination under deep anesthesia post-sampling.

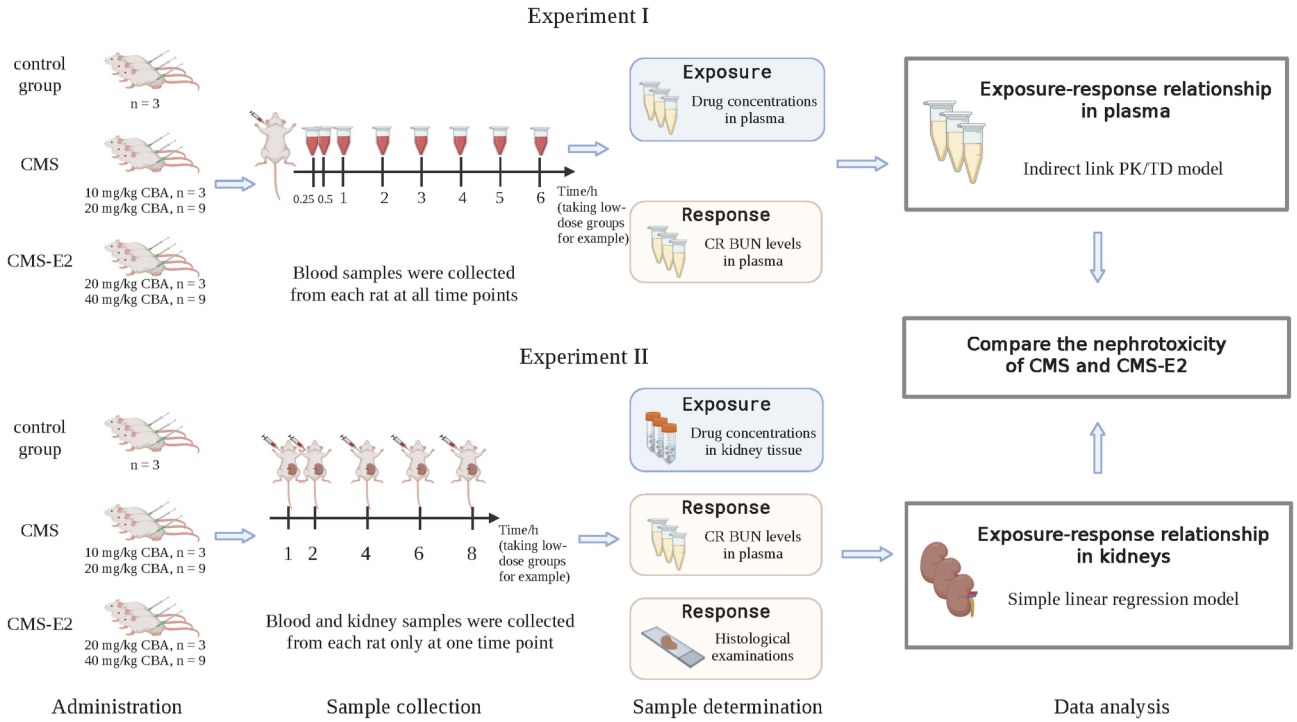

Fig. 6. The flow chart of study design. *CMS* colistin methanesulphonate, *CMS-E2* colistin B methanesulphonate, *CBA* colistin base activity, *CR*, creatinine, *BUN* blood urea nitrogen. Created with BioRender.com.

Reagents and materials

CMS (batch number: 190409115), CMS-E2 (batch number: 200117115) and polymyxin B1 sulfate (batch number: 20180516-B1) were supplied by Chiataitianqing Pharmaceutical Group Co. LTD (Jiangsu, China). Colistin sulfate (batch number: R046V0) was purchased from Pharmacopeial Convention, Inc (Maryland, USA). Teletamine-zolazepam hydrochloride was sourced from Virbac (Carros, France). Heparin sodium was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). HPLC-grade solvents including methanol, acetonitrile, and formic acid (50%) as well as ammonia (10%) were acquired from Sigma-Aldrich. Ultrapure water was generated using a Millipore Milli-Q system (Brussels, Belgium). Oasis WCX 96-well plates (10 mg/1 mL) and collection plates were purchased from Waters (Milford, MA, USA).

Study design and sample collection

The study comprised two experiments investigating PK-nephrotoxicity relationships, each containing one control and four treatment groups (Fig. 6). Doses were selected based on maximal/half-maximal toxicity thresholds in rats. Drugs were reconstituted in saline for intraperitoneal injection: CMS at 10/20 mg/kg CBA and CMS-E2 at 20/40 mg/kg CBA.

In experiment I, we explored plasma PK and nephrotoxicity biomarkers of CMS and CMS-E2. Intense blood samples were collected via jugular vein pre- and post-administration (Table S1). Blood aliquots (500 μL) were anticoagulated with heparin (1%), centrifuged (4,982 × g, 10 min, 4 °C), and stored at − 70 °C for plasma drug concentration, CR and BUN analyses.

In experiment II, the relationship between drug concentrations in kidneys and plasma nephrotoxicity biomarkers, as well as histological examination, were explored for CMS and CMS-E2. Rats in this group were anesthetized with tiletamine-zolazepam hydrochloride (40 mg/kg) and then sacrificed, followed by the collection of blood and bilateral kidney samples. Blood samples were used to determine the level of CR and BUN. The left kidney was immediately frozen and stored at − 70 °C for the quantification of drug concentrations. The right ones were fixed in 4% paraformaldehyde for 24 h for histological examination.

Analysis of CMS/CMS-E2 and colistin/colistin B concentrations in plasma and kidney

CMS/CMS-E2 and colistin/colistin B concentrations in plasma and renal tissues were quantified via liquid chromatography-tandem mass spectrometry (LC-MS/MS). Both plasma and kidney samples were divided into two aliquots for the determination of the prodrug and formed activity components. The formed colistin or colistin B concentrations were determined directly after sample treatment. The concentrations of CMS or CMS-E2 were measured indirectly as the difference between total colistin or colistin B concentrations and free colistin or colistin B concentrations. The total colistin or colistin B concentrations were determined by hydrolyzing all the prodrugs with sulfuric acid (0.5 mmol/L) for the conversion from CMS/CMS-E2 to colistin/colistin B.

The plasma CMS/CMS-E2 and colistin/colistin B concentrations were determined as described previously[41], and the determination of the concentrations in kidneys was slightly modified on this basis. Only the cortex was taken from the kidney samples for determination and added normal saline (1:10, m/v, containing 1% bovine serum albumin) for homogenization with a homogenizer (Precellys 24, Bertin Technologies LTD. France). For the sample treatment, solid phase extraction performed on Oasis WCX 96-well plates (10 mg/1 mL) (Waters, Milford, MA, USA) was employed. Either a 40 μL plasma sample or 200 μL kidney tissue homogenate was mixed with the internal standard of polymyxin B1. The mixture was loaded on a preconditioned WCX plate, and washed to remove interferences, then the eluents (30 μL) were injected into the LC-MS/MS. The mobile phase consisted of acetonitrile-methanol (v/v, 50:50, containing 0.1% formic acid) and 0.1% formic acid aqueous solution. The linear concentration range was 0.187–6.24 mg/L for colistin A, 0.037–1.23 mg/L for colistin B in plasma, and 0.0624–31.2 μg/g for colistin A, 0.0123–6.15 μg/g for colistin B in renal tissue. The inter-day precision and accuracy were within 10.0%, 7.3% for plasma 9.3%, and 9.9% for renal tissue, respectively.

Kidney injury biomarkers and renal histological examinations

Plasma CR and BUN were determined by an automatic biochemical analyzer (ADVIA Chemistry XPT System, Siemens Healthcare Diagnostics Inc. USA). The kidneys after paraffin embedding and sections were stained with PAS solution. Histological examinations were scored using a SQS, based on the degree of lesion and damage range score (Table S2).

PK analysis in plasma and kidney

NCA was performed using WinNonlin (version 8.1; Pharsight Corporation, USA). The following parameters were calculated for CMS, CMS-E2, and their formed activity components: V_d/F, CL/F, $t_{1/2}$ and AUC_{0-inf}.

PK-TD model for plasma concentrations and biomarkers

A compartmental model was developed for CMS and CMS-E2 following intraperitoneal injection. The model included an absorption compartment for CMS and CMS-E2, and one-, and two-compartment models were tested separately. An indirect link model with a linear-effect relationship was used to describe the PK-TD relationship between plasma drug concentrations and CR, BUN levels. This model is described by the equation: $E = E_0 + S \cdot C_e$, where E represents the plasm CR or BUN level; E_0 is the baseline level of CR or BUN prior to drug administration; S is the slope between colistin or colistin B concentrations in the effect compartment and CR or BUN levels; and C_e is the colistin or colistin B concentration in effect compartment. The goodness of fit was evaluated using the coefficient of determination (R2), Akaike information criterion (AIC), Bayesian information criterion (BIC), and visual examination of the experimental data. All models were developed using WinNonlin (version 8.1; Pharsight Corporation, USA).

Statistical analysis

Data were analyzed using Stata/SE 15.0 (StataCorp, College Station, TX, USA). As continuous variables, all PK and PK-TD parameter estimates are expressed as mean and standard deviation and compared by the Wilcoxon rank sum test. Simple linear regression and ordered logistic regression, which were used for the correlation analysis of a continuous variable and an ordered categorical variable, respectively, were applied to analyze the nephrotoxicity and drug concentrations in kidneys. Inter-group regression coefficients were compared using the Chow test[42]. $P < 0.05$ was considered statistically significant.

Data availability

Data is provided within the manuscript or supplementary information files.

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Author contributions

Chenxue Guo, Lin Xi, and Xingyi Qu performed experiments. Chenxue Guo, and Lin Xi conducted data acquisition and statistical analysis. Chenxue Guo wrote the manuscript. Zhiwei Huang, and Size Li provided software

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Declarations

Competing interests
The authors declare no competing interests.

Institutional review board statement
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Additional information
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Correspondence and requests for materials should be addressed to X.L. or J.Z.

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