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Preliminary Clinical Study of the Effect of Ascorbic Acid on Colistin-Associated Nephrotoxicity

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Nephrotoxicity is a dose-limiting factor of colistin, a last-line therapy for multidrug-resistant Gram-negative bacterial infections. An earlier animal study revealed a protective effect of ascorbic acid against colistin-induced nephrotoxicity. The present randomized controlled study was conducted in 28 patients and aimed to investigate the potential nephroprotective effect of intravenous ascorbic acid (2 g every 12 h) against colistin-associated nephrotoxicity in patients requiring intravenous colistin. Thirteen patients received colistin plus ascorbic acid, whereas 15 received colistin alone. Nephrotoxicity was defined by the RIFLE classification system. Additionally, urinary neutrophil gelatinase-associated lipocalin (NGAL) and *N*-acetyl-beta-D-glucosaminidase (NAG) were measured as markers of renal damage, and plasma colistin concentrations were quantified. The baseline characteristics, clinical features, and concomitant treatments of the patients in the two groups were comparable. The incidences of nephrotoxicity were 53.8% (7/13) and 60.0% (9/15) in the colistin-ascorbic acid group and the colistin group, respectively ($P = 0.956$; relative risk [RR], 0.9; 95% confidence interval, 0.47 to 1.72). In both groups, the urinary excretion rates of NGAL and NAG on day 3 or 5 of colistin treatment and at the end of colistin treatment were significantly higher than those at the respective baselines ($P < 0.05$). However, the urinary excretion rates of these biomarkers at the various times during colistin treatment did not differ significantly between the groups ($P > 0.05$). The plasma colistin concentrations in the two groups were not significantly different ($P > 0.28$). The clinical and microbiological outcomes and mortality of the patients in the two groups were not significantly different. This preliminary study suggests that ascorbic acid does not offer a nephroprotective effect for patients receiving intravenous colistin. (This study has been registered at ClinicalTrials.gov under registration no. NCT01501968.)

Infections caused by carbapenem-resistant Gram-negative bacteria, such as *Acinetobacter baumannii*, have become a serious problem globally, including in Thailand (1–3). Colistin (i.e., polymyxin E) and polymyxin B have been reintroduced over the past decade for therapy of multidrug-resistant (MDR) Gram-negative bacterial infections, especially those caused by carbapenem-resistant Gram-negative pathogens (4). Colistin is administered parenterally as colistimethate sodium (CMS), an inactive prodrug that is converted to colistin, the entity that possesses both antibacterial activity and the potential to cause toxicity (5, 6). The efficacy of colistin for treatment of infections by MDR Gram-negative bacteria is modest, and nephrotoxicity is the major dose-limiting factor in the clinical use of colistin (7, 8). The incidence of colistin-associated nephrotoxicity ranges from approximately 10% to 55%, and it appears to be related to the total dose of CMS, the duration of therapy, and the plasma colistin concentration, with a concentration higher than ~2.5 mg/liter associated with increased risk for nephrotoxicity (9–15). Colistin-associated nephrotoxicity usually occurs within the first 5 days of treatment and is reversible upon cessation of colistin (15, 16).

The detailed mechanism of polymyxin-induced nephrotoxicity remains unclear. The renal injury observed is primarily confined to the epithelium of proximal tubules (17). The propensity for polymyxins to cause nephrotoxicity is almost certainly related to the very extensive renal tubular reabsorption that has been demonstrated in both animals (18) and patients (19), thereby exposing these cells to a large load of polymyxins (20–22). The avid reabsorption may be attributed to numerous transporters located in the proximal tubules (20, 23). Megalin, encoded by a member of the low-density lipoprotein receptor gene family, is an endocytosis receptor expressed in the apical membranes of proximal tubular epithelial cells (24). It is evident that megalin plays a role in the uptake and accumulation of polymyxin B and colistin into renal cells (23, 25, 26). Recent studies have suggested that colistin-induced nephrotoxicity might be associated with oxidative stress (22, 27). With the oxidative stress caused by many other drugs, including gentamicin, cisplatin, and vancomycin, reactive oxygen species generated via mitochondria have been demonstrated to play an important role in tubular cell apoptosis leading to renal dysfunction (28–30). Ascorbic acid, a chain-breaking antioxidant and free radical scavenger (31–33), can attenuate renal damage in animals caused by a variety of insults, such as postischemic stress, gentamicin, and vancomycin (34–37). A recent study in rats showed the protective effect of coadministered ascorbic acid against colistin-induced nephrotoxicity and tubular apoptosis (38). The objective of this study was to determine the potential nephroprotective effect of ascorbic acid against colistin-associated

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nephrotoxicity in patients receiving intravenous CMS for treatment of infections by MDR Gram-negative bacteria. This report describes the outcomes of the interim analysis conducted after the study had enrolled half the planned number of subjects.

MATERIALS AND METHODS

Study design. The study was approved by the Siriraj Institutional Review Board, and informed consent was obtained from participating subjects or their legal guardians. This was an open-label, nonplacebo, randomized controlled study conducted at Siriraj Hospital, Bangkok, Thailand, during the period December 2011 to February 2013. The study has been registered at ClinicalTrials.gov under registration no. NCT01501968.

Patient population. The study subjects were adult (aged ≥18 years) medical patients with infections by MDR Gram-negative bacteria who required colistin therapy. All patients had pneumonia or tracheobronchitis, and one patient with pneumonia also had bacteremia. The exclusion criteria were as follows: pregnancy or breastfeeding, known allergy to ascorbic acid and/or colistin, baseline serum creatinine level of ≥2 mg/dl, hypotension at enrollment, receiving other nephrotoxic drugs at enrollment (e.g., aminoglycosides, vancomycin, or amphotericin B), anticipated administration of contrast medium within 7 days, underlying diseases (e.g., cancer, HIV infection, systemic lupus erythematosus, glucose-6-phosphate-dehydrogenase deficiency, or urinary tract stone), or unlikelihood of receiving the study medications for at least 72 h.

Interventions. The eligible patients were assigned to the colistin group or the colistin-ascorbic acid group by block randomization. CMS was administered intravenously at a loading dose of 300 mg of colistin base activity (CBA) (this dose is equivalent to ~10 million IU) followed by renally adjusted maintenance doses given every 12 h in both groups. Ascorbic acid was administered intravenously at a dose of 2 g every 12 h 20 min before CMS in the patients randomized to the colistin-ascorbic acid group. The treating physician determined the duration of the CMS regimen, and ascorbic acid was discontinued at the same time as CMS.

Serum creatinine concentrations were measured prior to colistin treatment (baseline) and on days 3 and 5, at the end of treatment, and at additional times as needed. The creatinine clearance was calculated by the Cockcroft-Gault formula using actual body weight (39–41).

Urine samples were collected at baseline (day1) and during and at the end of colistin treatment. Each urine sample was centrifuged at 1,500 × *g* for 15 min at 4°C, and the supernatant was stored at −80°C. The urine samples collected prior to colistin treatment (baseline), on day 3 (if the subject developed acute kidney injury [AKI] on or before day 5) or day 5 (if the subject developed AKI at a later time or did not develop AKI), and at the end of colistin treatment were subjected to analysis for neutrophil gelatinase-associated lipocalin (NGAL) and *N*-acetyl-beta-D-glucosaminidase (NAG). The urinary NGAL concentration was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Bioporto, Denmark; no. 036). The urinary NAG concentration was measured by colorimetric assay using a commercially available kit (Roche Applied Science, Indianapolis, IN, USA) according to the manufacturer's protocol. The measurements were made in duplicate and in a blinded fashion.

Blood samples were collected at 0.5 and 4 h after the end of the CMS infusion and prior to the next dose of CMS from each subject on days 1, 2, and 7 or the last day of colistin treatment if colistin was discontinued before day 7. Heparinized blood collection tubes were used, and blood samples were placed on ice immediately and centrifuged at 4°C within 1 h of collection. Plasma samples were stored at −80°C to prevent *in vitro* conversion of CMS to colistin (42). Plasma colistin concentrations were measured within 4 months of collection at Monash Institute of Pharmaceutical Sciences, Monash University (Melbourne, Australia), using high-performance liquid chromatography (43).

Data collection and outcome measurements. The clinical features, clinical courses, dose and treatment duration of CMS and concomitant nephrotoxic agents, urine output, and clinical response to treatment were collected. Nephrotoxicity or AKI was determined according to the RIFLE classification (39) based on changes in the serum creatinine concentration from baseline to the peak value. The outcome in regard to the treatment of the index infection was classified clinically and microbiologically. The clinical outcomes were classified as favorable outcome (cure or improvement of signs and symptoms of the index infection) or nonfavorable outcome (persistence or progression of the index infection or death). Microbiological outcomes were classified as eradication or persistence of index pathogens or as undetermined if the relevant clinical specimen was not collected or the specimen was not a true respiratory specimen determined by the microbiology laboratory.

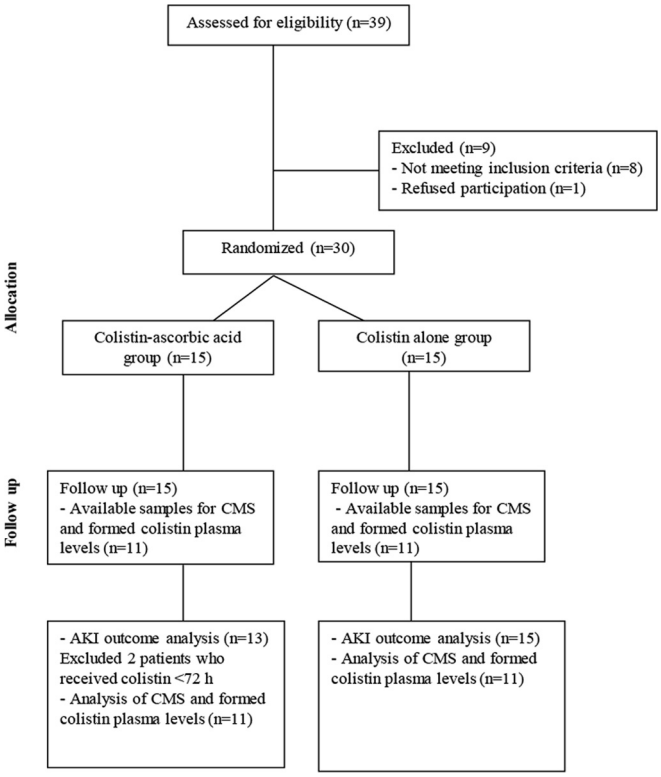

FIG 1 Flow of the study.

Statistical analyses. A sample size of 54 patients across the two groups was estimated to be needed based upon the assumption that the colistin-associated nephrotoxicity rate was 50% in the colistin group and 15% in the colistin-ascorbic acid group, with 5% type I error and 20% type II error. However, an interim analysis was planned to be performed when 50% of the subjects had been enrolled. The demographic information, medical history, laboratory data, treatments, incidence of AKI, and excretion of urinary NGAL and NAG were expressed in percentages, means, standard deviations (SD), and medians, as appropriate. The comparisons of characteristics and outcomes between the two groups were performed using a chi-square test or Fisher's exact test for categorical data and Student's *t* test or the Mann-Whitney U test for continuous data. All reported *P* values were two sided. A *P* value of 0.05 or less was considered statistically significant. All statistical analyses were performed using Stata statistical software version 10.0 (Stata Corporation, College Station, TX, USA).

RESULTS

Outline of the study. Thirty-nine patients were assessed for eligibility, and 30 patients were enrolled, as shown in Fig. 1. However, two enrolled patients were excluded from analysis because the duration of colistin therapy was less than 72 h. Therefore, 28 pa-

TABLE 1 Characteristics of patients by treatment group

Characteristic	Value for treatment group: Colistin-ascorbic acid ($n = 13$)	Colistin ($n = 15$)	*P* value
Male [*n* (%)]	7 (53.8)	5 (33.3)	0.274
Mean (SD) age (yr)	76.4 (12.5)	68.9 (20.5)	0.564
Mean (SD) body weight (kg)	49.8 (5.5)	52.3 (8.7)	0.480
Underlying disease [*n* (%)]			
Diabetes mellitus	3 (23.1)	4 (26.7)	1.000
Hypertension	3 (23.1)	8 (53.3)	0.137
Cardiovascular disease	3 (23.1)	6 (40.0)	0.435
Chronic lung disease	6 (46.2)	4 (26.7)	0.433
Chronic kidney disease	0 (0)	1 (6.7)	1.000
Cirrhosis	2 (15.4)	0 (0)	0.115
Stroke	5 (38.5)	6 (40.0)	0.934
Epilepsy	0 (0)	1 (6.7)	1.000
Ward at enrollment [*n* (%)]			0.228
ICU	6 (46.2)	3 (20.0)	
General	7 (53.8)	12 (80.0)	
APACHE II score			0.756
Mean (SD)	16.2 (4.7)	16.8 (4.9)	
Median	16	17	
Site of infection [*n* (%)]			0.564
Pneumonia	7 (53.8)	10 (66.7)	
Tracheobronchitis	5 (38.5)	5 (33.3)	
Pneumonia + bacteremia	1 (7.7)	0 (0)	
Causative bacterium [*n* (%)]			
Acinetobacter baumannii	13 (100.0)	12 (80.0)	0.226
Pseudomonas aeruginosa	2 (15.4)	7 (46.7)	0.114
Klebsiella pneumoniae	2 (15.4)	0 (0)	0.206
Concomitant antibiotic during colistin therapy [*n* (%)]			
Carbapenem	8 (61.5)	8 (53.3)	0.718
Piperacillin-tazobactam	0 (0)	2 (13.3)	0.172
Sulbactam	1 (7.7)	1 (6.7)	1.000
Fosfomycin	0 (0)	1 (6.7)	1.000
Levofloxacin	2 (15.4)	3 (20.0)	1.000
Vancomycin	1 (7.7)	1 (6.7)	1.000
CBA administration			
Mean (SD) duration of CMS (days)	9.4 (3.1)	10.1 (5.8)	0.835
Median duration of CMS (days)	9	8	
Mean (SD) total dose of CBA (mg)	1,923 (509)	2,263 (1221)	0.908
Median total dose of CBA (mg)	1,950	1,800	
Mean (SD) dose of CBA per day (mg)	216 (62.8)	235 (55.8)	
Median dose of CBA per day (mg)	207	217	0.395
Mean (SD) dose CBA per kg body weight/day	4.4 (1.3)	4.6 (1.3)	
Median dose CBA per kg/day	4.2	4.7	0.637
Total fluid intake per day (ml)			
Mean (SD)	2,626 (636)	2,627 (819)	0.998
Median	2,567	2,465	
Total urine output per day (ml)			
Mean (SD)	1,533 (778)	1,633 (723)	0.726
Median	1,646	1,384	
Presence of hypotension [*n* (%)]	6 (46.2)	7 (46.7)	0.978

(Continued on following page)

TABLE 1 (Continued)

Characteristic	Value for treatment group: Colistin-ascorbic acid ($n = 13$)	Colistin ($n = 15$)	*P* value
Duration of hypotension (days)			
Mean (SD)	2.2 (1.3)	2.6 (2.2)	1.000
Median	2	2	
Vasopressor drug use [*n* (%)]	2/6 (33.3)	5/7 (71.4)	0.286
Serum creatinine concn (mg/dl) at baseline			
Mean (SD)	0.8 (0.4)	0.9 (0.5)	0.660
Median	0.7	1	
Urinary NGAL excretion (μg/h) at baseline			
Mean (SD)	37.7 (30.0)	32.6 (32.1)	0.669
Median	30.2	18.0	
Urinary NAG excretion (μg/h) at baseline			
Mean (SD)	2.5 (2.2)	2.9 (2.8)	0.755
Median	1.8	1.4	

tients (13 in the colistin-ascorbic acid group and 15 in the colistin group) were evaluated in the intention-to-treat analyses. In 6 subjects in the colistin-ascorbic acid group, due to sepsis that required immediate initiation of colistin therapy, it was necessary to administer the first dose of ascorbic acid simultaneously with colistin; different intravenous lines were used to prevent potential drug interaction and instability.

Characteristics of the study subjects. The baseline characteristics, clinical features, and treatments of the patients in the two groups were comparable (Table 1). The patients in the colistin-ascorbic acid group were somewhat older. Six patients (46.2%) and three patients (20%) in the colistin-ascorbic acid group and the colistin group, respectively, were in the intensive care unit (ICU) at enrollment. The mean APACHE II (Acute Physiology and Chronic Health Evaluation II) score, a severity-of-disease classification system, was not significantly different between the groups. Pneumonia was the most common infection, and the most common causative bacterial pathogen was *A. baumannii*. The majority of patients received concomitant antimicrobial therapy. One patient in the colistin-ascorbic acid group received vancomycin on day 2 of colistin treatment, and one patient in the colistin group also received vancomycin on day 4 of colistin treatment. The mean daily doses of CBA administered in the colistin-ascorbic acid and colistin groups were 216 and 235 mg, respectively, and the corresponding mean cumulative doses of CBA were 1,923 and 2,263 mg. The mean durations of colistin treatment in the colistin-ascorbic acid group and the colistin group were 9.4 and 10.1 days, respectively. The total daily fluid intake and urine output, presence of hypotension, and use of vasoactive agents were similar in the two groups. At baseline, the serum creatinine concentrations and urinary excretion of NGAL and NAG were not significantly different between the two groups.

Outcomes of the study subjects. The serum creatinine concentration and clearance of creatinine at baseline and during colistin treatment and AKI classified according to the RIFLE criteria are shown for both groups in Table 2. The serum creatinine concentrations and creatinine clearance values at various times of determination and the absolute changes from baseline values in the two groups were not significantly different. The serum creatinine concentrations of both groups peaked after 6 to 8 days of colistin therapy. The incidences of AKI, according to the RIFLE criteria (39), were 53.8% and 60.0% in the colistin-ascorbic acid and colistin groups, respectively ($P = 0.956$; relative risk [RR], 0.9; 95% confidence interval, 0.47 to 1.72). The colistin-ascorbic acid group tended to have a category of "failure" in RIFLE criteria more than the colistin group.

The urinary excretion rates of NGAL and NAG for all patients at various collection times are shown in Table 3. The excretion rates of NGAL and NAG at baseline, on day 3 or 5 of colistin treatment, and at the end of colistin treatment were not significantly different between the colistin-ascorbic acid group and the colistin group. In each group, urinary excretion of NGAL and NAG on day 3 or 5 of colistin treatment and at the end of colistin treatment was significantly higher than the corresponding baseline value ($P < 0.05$). The urinary excretion rates of both nephrotoxicity biomarkers at various collection times in the patients with AKI are shown in Table 4. Even though the rates of NGAL and NAG urinary excretion on day 3 or 5 of colistin treatment and at the end of colistin treatment were significantly higher than the corresponding baseline value in each group ($P < 0.05$), no significant difference was observed between the groups. Urinary NGAL and NAG excretion rates were not significantly different between patients with and without AKI either at baseline or at the end of colistin treatment ($P > 0.1$).

A total of 163 plasma samples from 22 patients (11 in each group) were available for measurement of colistin concentrations; on the third day of collection (day 7 or the last day of treatment), samples were available from only 6 and 7 patients in the colistin and colistin-ascorbic acid groups, respectively. The plasma concentrations of colistin in the colistin-ascorbic acid group were not significantly different from those in the colistin group ($P > 0.28$ for each of the three time points on days 1, 2, and 7 [or the last day] of colistin treatment).

The clinical and microbiological outcomes and mortality for

TABLE 2 Creatinine data and classification of AKI according to the RIFLE criteria for the patients in each treatment group

Outcome	Value for treatment group: Colistin-ascorbic acid ($n = 13$)	Colistin ($n = 15$)	*P* value
Serum creatinine (SCr) concn (mg/dl)			
Baseline			
Mean (SD)	0.8 (0.4)	0.9 (0.5)	0.660
Median	0.7	1	
Peak			
Mean (SD)	2.2 (2.2)	1.8 (0.9)	0.782
Median	1.6	1.9	
End of treatment			
Mean (SD)	1.7 (1.8)	1.7 (0.9)	0.344
Median	1.4	1.8	
Change in SCr (peak − baseline)			
Absolute (mg/dl) [mean (SD)]	1.4 (2.0)	0.8 (0.6)	0.854
Median	0.8	0.7	
Relative to baseline (%)[mean (SD)]	181 (213)	102 (101)	0.210
Creatinine clearance (CrCl) (ml/min)			
Baseline			
Mean (SD)	56.8 (22.2)	76.4 (78.8)	0.662
Median	49.4	44.6	
Lowest			
Mean (SD)	31.0 (21.0)	52.5 (77.2)	0.908
Median	25.0	28.2	
End of treatment			
Mean (SD)	33.8 (20.6)	54.2 (77.0)	0.730
Median	26.6	28.2	
Change in CrCl (lowest − baseline)			
Absolute (ml/min) [mean (SD)]	−25.8 (24.5)	−23.9 (20.5)	0.836
Median	−19.4	−21.9	
Relative to baseline (%)[mean (SD)]	−47.9 (33.0)	−41.2 (20.4)	0.518
Day of peak SCr after commencing colistin treatment			
Mean (SD)	6.8 (3.2)	8.1 (6.0)	0.963
Median	6	6	
AKI (RIFLE criteria) [*n* (%)]			
Risk	0 (0)	2 (13.3)	0.484
Injury	2 (15.4)	6 (40.0)	0.221
Failure	5 (38.5)	1 (6.7)	0.069
All	7 (53.8)	9 (60.0)	0.956

TABLE 3 Urinary excretion of NGAL and NAG for all patients by treatment group

Outcome[a]	Value for treatment group: Colistin-ascorbic acid ($n = 13$)[b]	Colistin ($n = 15$)[b]	*P* value
Urinary NGAL excretion (μg/h)			
Baseline [mean (SD)]	37.7 (30.0)	32.6 (32.1)	0.669
D3/5 [mean (SD)]	71.8 (37.4)	66.2 (55.1)	0.759
ET [mean (SD)]	73.8 (41.8)	74.5 (60.2)	0.972
Change in urinary NGAL (D3/5 − baseline)			
Absolute (μg/h) [mean (SD)]	31.3 (42.1)	32.3 (47.9)	0.954
Relative to baseline (%)[mean (SD)]	348 (718)	422 (754)	0.795
Change in urinary NGAL (ET − baseline)			
Absolute (μg/h) [mean (SD)]	33.0 (41.1)	46.7 (49.9)	0.440
Relative to baseline (%)[mean (SD)]	317 (577)	478 (773)	0.543
Urinary NAG excretion (μg/h)[c]			
Baseline [mean (SD)]	2.5 (2.2)	2.9 (2.8)	0.755
D3/5 [mean (SD)]	7.6 (5.3)	8.5 (10.8)	0.836
ET [mean (SD)]	7.1 (6.5)	12.7 (14.5)	0.336
Change in urinary NAG (D3/5 − baseline)[c]			
Absolute (μg/h) [mean (SD)]	5.8 (5.9)	5.6 (9.8)	0.961
Relative to baseline (%)[mean (SD)]	500 (498)	324 (607)	0.538
Change in urinary NAG (ET − baseline)[c]			
Absolute (μg/h) [mean (SD)]	4.8 (6.7)	9.8 (15.1)	0.406
Relative to baseline (%)[mean (SD)]	382 (331)	1,181 (2,576)	0.399

[a] D, day of colistin treatment; D3/5, D3 or D5; ET, end of treatment.
[b] Unless otherwise indicated.
[c] $n = 8$ for both groups.

the patients in the two groups (Table 5) were not significantly different ($P > 0.5$). No adverse events related to ascorbic acid were observed.

DISCUSSION

The present study aimed to determine if ascorbic acid, which was found to protect against colistin-induced nephrotoxicity in rats (38), could have a nephroprotective effect against colistin-associated nephrotoxicity in patients. Ascorbic acid at 4 g/day was chosen for this study because such a dosage was reported to be safe without increased urinary oxalate (44, 45) and a similar dose was found to be effective in a previous clinical study for preventing nephrotoxicity after contrast medium injection (46).

In this study, the overall incidence of nephrotoxicity, determined according to the widely used RIFLE criteria (39), was 50 to 60%, and it was not significantly different from that observed in the patients at Siriraj Hospital in 2007 (47). The incidence of AKI in the colistin-ascorbic acid group (53.8%) was not significantly different from that in the colistin group (60.0%). The lack of nephroprotection, based upon use of the RIFLE criteria, was supported by the results for the urinary excretion of the biomarkers NGAL and NAG. Although serum creatinine is widely used to monitor kidney function and is a key component when AKI is assessed, NGAL can be used as a marker of kidney injury (48) and NAG is a widely used biomarker of renal tubular dysfunction (49).

TABLE 4 Urinary excretion of NGAL and NAG for patients with AKI by treatment group

Outcome[a]	Value for treatment group: Colistin-ascorbid acid ($n = 7$)	Colistin ($n = 9$)[b]	*P* value
Urinary NGAL excretion (μg/h)			
Baseline [mean (SD)]	57.2 (35.6)	31.6 (24.6)	0.111
D3/5 [mean (SD)]	73.2 (48.1)	56.3 (51.7)	0.515
ET [mean (SD)]	79.7 (58.5)	52.3 (50.9)	0.334
Change in urinary NGAL (D3/5 − baseline)			
Absolute (μg/h) [mean (SD)]	20.7 (46.0)	21.9 (40.1)	0.956
Relative to baseline (%) [mean (SD)]	64.3 (134.8)	352 (803)	0.368
Change in urinary NGAL (ET − baseline)			
Absolute (μg/h) [mean (SD)]	21.8 (55.7)	18.0 (38.1)	0.873
Relative to baseline (%) [mean (SD)]	45.7 (111)	223 (492)	0.370
Urinary NAG excretion (μg/h)[c]			
Baseline [mean (SD)]	3.5 (2.2)	2.5 (3.0)	0.481
D3/5 [mean (SD)]	7.9 (6.6)	10.8 (16.5)	0.671
ET [mean (SD)]	8.2 (8.1)	9.3 (8.5)	0.802
Change in urinary NAG (D3/5 − baseline)[c]			
Absolute (μg/h) [mean (SD)]	5.6 (7.8)	8.3 (13.5)	0.650
Relative to baseline (%) [mean (SD)]	282 (338)	262 (186)	0.885
Change in urinary NAG (ET − baseline)[c]			
Absolute (μg/h) [mean (SD)]	5.1 (8.7)	6.8 (5.8)	0.659
Relative to baseline (%) [mean (SD)]	316 (436)	335 (212)	0.912

[a] D, day of colistin treatment; D3/5, D3 or D5; ET, end of treatment.
[b] Unless otherwise indicated.
[c] $n = 8$ for the colistin group.

TABLE 5 Clinical and microbiological outcomes of the patients by treatment group

Outcome	Value for treatment group: Colistin-ascorbic acid ($n = 13$)	Colistin ($n = 15$)	*P* value
Clinical outcome at day 3 [*n* (%)]			0.685
Cured	0 (0)	1 (6.7)	
Improved	7 (53.8)	6 (40.0)	
Unchanged	5 (38.5)	8 (53.3)	
Dead from infection	0 (0)	0 (0)	
New infection	0 (0)	0 (0)	
Nonevaluable	1 (7.7)	0 (0)	
Microbiological outcome at day3 [*n* (%)]			0.105
Eradication	3 (23.1)	1 (6.7)	
Persistence	1 (7.7)	6 (40.0)	
Undetermined	9 (69.2)	8 (53.3)	
Clinical outcome at end of treatment [*n* (%)]			1.000
Cured	10 (76.9)	11 (73.3)	
Improved	0 (0)	0 (0)	
Unchanged	0 (0)	1 (6.7)	
Dead from infection	2 (15.4)	3 (20.0)	
New infection	1 (7.7)	0 (0)	
Microbiological outcome at end of treatment [*n* (%)]			0.188
Eradication	8 (61.5)	5 (33.3)	
Persistence	3 (23.1)	3 (20.0)	
Undetermined	2 (15.4)	7 (46.7)	
Mortality			
At end of treatment [*n* (%)]	3 (23.1)	3 (20.0)	1.000
On day 28 [*n* (%)]	7 (53.8)	7 (46.7)	0.705

As colistin is extensively reabsorbed by renal tubular cells and causes apoptosis (18, 50), urinary NGAL and NAG excretion was monitored in the present study. Under normal conditions, urinary excretion of NGAL and NAG is low, but the excretion rises sharply from the respective basal levels in response to kidney injury to reach diagnostic levels within a very short time, as much as 24 h or more before any significant rise in serum creatinine (51–57). In the current study, the rates of urinary excretion of NGAL and NAG on day 3 or 5 of colistin treatment and at the end of treatment, in all patients as well as those with AKI as defined by the RIFLE system, were significantly higher than the respective baseline values in both the colistin and the colistin-ascorbic acid groups. However, no significant difference was observed between the groups in the excretion of NGAL and NAG at any of the time points during colistin treatment. Collectively, our results on the proportion of patients developing AKI and on the urinary excretion of the sensitive biomarkers suggest a lack of efficacy of ascorbic acid for preventing colistin-associated nephrotoxicity in the study patients.

There are several possible reasons for not observing nephroprotection in the patients who received ascorbic acid, even though it was effective in rats (38). First, the sample size of 28 patients may not be sufficient to detect a difference in the incidence of nephrotoxicity. The absolute difference in the incidence of nephrotoxicity (6.2%) observed in 28 enrolled patients is much less than that used in the sample size estimation (35%). For an absolute difference of 6.2% in the incidence of nephrotoxicity between the colistin-ascorbic acid and colistin groups to be detected, a sample size of at least 800 patients per treatment arm would be needed. Even if a study of this size was able to demonstrate a difference of 6.2%, the clinical significance of such a small effect is questionable. Second, there is increasing evidence that the risk of colistin-associated nephrotoxicity increases with the plasma colistin concentration (14, 15). Therefore, nephroprotection by ascorbic acid may have gone undetected if plasma colistin concentrations were higher in that group. However, the concentrations of colistin were not different between the groups. Third, it may be suggested that the dose of ascorbic acid in the current study was insufficient to elicit the

desired effect. It should be noted that the ascorbic acid dose used in the present study was similar to that found to be effective in preventing nephrotoxicity in patients receiving injections of contrast medium (46). Furthermore, after consideration of animal scaling (58), the daily dose of ascorbic acid (4 g per day) in the present study was similar to, or higher than, that used in the previous study in otherwise healthy rats in which nephroprotection was observed (38). Fourth, it is important to recognize the relative pathophysiological status and homogeneity of the rats in the preclinical study (38) and the patients in the present study. The subjects in the current study were a diverse population of infected patients with potentially confounding influences. Although all the studied patients had initial serum creatinine levels of less than 2 mg/dl and had no confounding conditions at enrollment likely to affect nephrotoxicity, the mean ages of the patients in both groups were relatively high (Table 1). In addition, during the study period, some patients experienced hypotension or received concomitant nephrotoxic agents (such as vancomycin) that may have predisposed them to develop acute renal insufficiency. A systematic review of comparisons of treatment effects between animal experiments and clinical trials revealed that discordant results might be due to the failure of animal models to mimic clinical disease adequately (59).

In conclusion, based upon the interim analysis of this randomized controlled study, coadministration of ascorbic acid was not shown to ameliorate colistin-associated nephrotoxicity. Neither the incidence of AKI, as assessed by the RIFLE criteria, nor the urinary excretion of the renal-injury biomarkers NGAL and NAG provided an indication of nephroprotection by ascorbic acid. Consequently it was considered inappropriate to proceed with the study. Thus, the addition of ascorbic acid to colistin therapy is not recommended in routine clinical practice. Close monitoring of renal function should be performed during colistin therapy.

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