Original Article

Evaluation of the Effectiveness of N-Acetylcysteine in the Prevention of Colistin-Induced Nephrotoxicity: A Randomized Controlled Clinical Trial

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ABSTRACT

Objective: The present study aimed to evaluate the effectiveness of N-Acetylcysteine (NAC), as an antioxidant, in preventing nephrotoxicity in patients receiving colistin. **Methods:** In a randomized controlled clinical trial, eligible participants receiving colistin were divided into two groups including drug (n = 43) and control (n = 39). In the drug group, 1200 mg of NAC was administered daily for 10 days concurrently with colistin. Patients in the control group received only colistin. The serum creatinine level (SCr), blood urea nitrogen (BUN), and creatinine clearance (CrCl) at baseline and every other day, and the number of cases with acute kidney injury (AKI) during the study were recorded. Before starting treatment and on day 5, the level of urinary neutrophil gelatinase-associated lipocalin (NGAL) was determined. Finally, the values were compared between the groups. **Findings:** There was a significant increase in SCr and BUN and a significant reduction in CrCl in both groups, but there was not any significant difference between the two groups at any time. Changes in the urine NGAL levels were not significantly different between the two groups. Even though the number of cases with AKI in the drug group (8 cases, 18.6%) was less than the control group (11 cases, 28.2%), the difference was not statistically significant (P = 0.303). **Conclusion:** Simultaneous administration of NAC with a dose of 1200 mg daily does not have any effect in the prevention of colistin-induced nephrotoxicity.

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INTRODUCTION

Colistin (polymyxin E) is a cationic polypeptide antibiotic that is used as the last treatment of multidrug-resistant Gram-negative bacteria, including *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.[1] The pharmacokinetic and pharmacodynamic studies indicate that the currently prescribed dose of colistin for critically ill patients does not increase the plasma concentration of this drug to a sufficient level, and a higher dose is necessary to prevent resistance and achieve the desired bactericidal effect;[2] however,

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nephrotoxicity is a major colistin dose-limiting factor. Therefore, there is a fundamental need to find a way to reduce the nephrotoxicity of colistin.

The incidence of nephrotoxicity due to colistin is about 20% to 60%[3] and seems to be due to the total (cumulative) dose of the drug, the serum concentration of the drug, and the treatment duration; concentrations over 2.5 mg/L lead to a higher risk of nephrotoxicity.[4,5] Nephrotoxicity of colistin usually occurs within the first five days of treatment and can lead to acute renal failure. Even though nephrotoxicity is reversible by discontinuation of colistin, limiting the dose of colistin is the most important complication.[5]

The mechanism of colistin-induced nephrotoxicity is not fully understood. Recent studies indicate that oxidative stress plays a main role in inducing this adverse effect. Colistin increases the number of reactive oxygen species that play an important role in causing renal tubular cell apoptosis and renal dysfunction.[6] It has been reported that colistin decreases superoxide dismutase 2 (SOD2) and endothelial nitric oxide synthase (eNOS). It also decreases the level of glutathione, the main body antioxidant protecting cells against oxidative stress.[7,8]

Due to the involvement of oxidative reactions in the pathogenesis of colistin nephrotoxicity, antioxidant agents possibly have the potential to reduce this complication. Animal studies have indicated the beneficial effects of some antioxidants such as melatonin,[9] Vitamins E and C,[10] lycopene,[11] and astaxanthin[12] in reducing colistin-induced kidney injury.

N-acetylcysteine (NAC) is a precursor in the formation of glutathione in the body. It has antioxidant and free radical scavenging effects.[13] NAC can exert its antioxidant effect directly and react with electrophilic groups of free radicals through its thiol group.[14]

Studies have indicated that NAC decreases the nephrotoxic effects of vancomycin,[15] cisplatin,[16] and gentamicin.[17] An animal study also revealed that concomitant administration of NAC with colistin reduces the oxidative stress caused by colistin in kidney cells;[18] however, there is not any prospective clinical trial in this field. Therefore, the present study aimed to clinically investigate the possible effectiveness of NAC in the prevention of this complication.

Methods

A randomized controlled clinical trial was conducted in Al-Zahra hospital and the Faculty of Pharmacy, which were both affiliated to Isfahan University of Medical Sciences (IUMS), from September 2019 to July 2021. The research protocol was registered in the Iranian Registry of Clinical Trials (IRCT) with a code of IRCT 20150721023282N6.

Patients treated with colistin for any reason in different wards of the hospital were selected. Inclusion criteria were as follows: (1) age of 12 years and above, (2) receiving colistin at a dose of 4.5 million units every 12 h, and (3) creatinine clearance (CrCl) >90 ml/min.

Exclusion criteria were (1) any kidney disorder including glomerulonephritis, polycystic kidney disease, kidney stones, interstitial nephritis, renal artery stenosis, and renal carcinoma (based on the medical history); (2) underlying diseases causing kidney dysfunction such as diabetes mellitus and hypertension (based on the medical history); (3) history of acute kidney injury (AKI) (based on the medical history); (4) receiving other nephrotoxic drugs such as aminoglycosides, amphotericin B, cyclosporine, tacrolimus, furosemide, vancomycin, iodinated contrast agents, cisplatin, and nonsteroidal anti-inflammatory drugs; (5) taking other antioxidant supplements such as Vitamins C and E; and (6) history of has been to NAC.

All patients were interviewed before entering the study to get acquainted with the project. Written consent was obtained from all participants. The research ethics committee of IUMS also approved the research protocol with an ethical code of IR.MUI.RESEARCH.REC.1398.361.

Individuals who took colistin with the dose of 150 mg (equivalent to 4.5 million units of colistimethate sodium) every 12 h for any reason and met other inclusion criteria were randomly divided into drug (NAC) and control groups. The block randomization method was utilized for randomization. To this end, blocks of four were used and the patients were divided into two groups based on the sequences specified in the randomly selected blocks. The demographic and clinical characteristics of patients were recorded, including age, gender, and diagnosis (indication for colistin use). The NAC effervescent tablets at a dose of 600 mg were prescribed twice daily for the patients in the drug group simultaneously with colistin for 10 days, while the patients in the control group received only colistin. Patients were excluded from the study if colistin was discontinued for reasons other than AKI. In case of AKI, it was decided to discontinue or continue treatment with colistin based on the medical team's opinion.

Blood samples were taken from patients in both groups before the treatment, during the treatment every

other day, and also 12 h after the last dose of colistin on the 10th day of treatment with this antibiotic to determine serum creatinine (SCr) levels and blood urea nitrogen (BUN) as well as CrCl; the latter was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[19] Furthermore, the levels of urinary neutrophil gelatinase-associated lipocalin (NGAL) were measured and recorded as an index for rapid detection of AKI for all patients before the study and on the fifth day of treatment (before administration of a dose of colistin). To determine AKI and its stages, Kidney Disease: Improving Global Outcomes (KDIGO) definition and classification of AKI were used as follows: stage 1, an increase in SCr equal to 0.3 mg/dl or more within 48 h or an increase in SCr≥1.5–2 times the baseline (initial) value within 7 days; Stage 2, an increase in SCr≥2–3 times the baseline value within 7 days; and Stage 3, an increase in SCr≥3 times the baseline value within 7 days.[20]

The means of SCr, BUN, CrCl, and urinary NGAL at the measured times and also the incidence of AKI in terms of the number of cases per stage were compared between the drug and control groups.

To determine the urinary levels of NGAL, fresh morning urine samples were taken from the patients and centrifuged for 20 min (2000 rpm) and stored in the freezer at −70°C. At the end of the study, all samples were taken out of the freezer, and after thawing at room temperature, the concentration of urine NGAL was measured using enzyme-linked immunoassay kits (R&D Systems Company, USA) according to the manufacturer instructions.

Outcome variables included the changes of SCr, BUN, and CrCl on the 2nd, 4th, 6th, 8th, and 10th days of treatment, and the changes in the urinary NGAL levels on the 5th day of treatment compared to baseline, as well as the number of AKI cases both totally and separately by the stage during the intervention.

According to the main variable of the project (CrCl), which was a continuous quantitative type, the following formula was used to calculate the sample size:

$$n = \frac{(Z_{1-\alpha/2} + Z_{\beta})^2 \times 2 \times (SD)^2}{(d)^2}$$

Error α was 5%, error β was 20%, and d (the minimum significant difference between the two groups) was 10 according to a similar study on cisplatin nephrotoxicity. According to the same study, the standard deviation of CrCl was 14;[21] hence, 30 patients were considered for each study group.

For statistical analysis, SPSS software version 24 (SPSS Inc., Chicago, USA) was used. Independent samples *t*-test was used to compare the values of parameters at any time, and the repeated-measures ANOVA was employed to compare the values within each group. Chi-square and Fisher's exact tests were utilized to compare the qualitative parameters between the two groups. $P < 0.05$ was considered statistically significant.

Results

In the study, 219 patients were evaluated in terms of eligibility; 111 patients met the inclusion criteria and entered the study. Furthermore, 13 and 16 patients were

Table 1: Basic demographic and clinical information of the patients

Parameter	Drug group, *n* (%)	Control group, *n* (%)	*P*
Gender			
Male	29 (67.4)	26 (67.7)	0.941
Female	14 (32.6)	13 (33.3)	
Age	51.49	47.82	0.294
Ward			
ICU	37 (86.1)	34 (87.1)	0.717
Surgery	1 (2.3)	1 (2.6)	
Neurology	4 (9.3)	2 (5.1)	
Infectious diseases	1 (2.3)	1 (2.6)	
Urology and hematology	0	1 (2.6)	
Comorbidity	23	13	
DM	0	1 (7.7)	0.336
HTN	9 (39.1)	3 (23.1)	
Hyperthyroidism	0	1 (7.7)	
Epilepsy	2 (8.7)	0	
Brain tumor	1 (4.3)	0	
Laryngeal cancer	1 (4.3)	0	
IHD	1 (4.3)	0	
HLP	1 (4.3)	0	
Rheumatoid arthritis	0	2 (15.4)	
Asthma	0	1 (7.7)	
HTN + DM	2 (8.7)	3 (23.1)	
HTN + heart failure	0	1 (7.7)	
HTN + IHD	1 (4.3)	0	
DM + HLP	1 (4.3)	1 (7.7)	
IHD + stroke	1 (4.3)	0	
HTN + IHD + DM	1 (4.3)	0	
HTN + IHD + HLP	1 (4.3)	0	
Diagnosis			
Ventilator-associated pneumonia	35 (81.4)	31 (79.5)	0.584
Meningitis	4 (9.3)	6 (15.4)	
Aspiration pneumonia	2 (4.7)	1 (2.6)	
Pyelonephritis	0	1 (2.6)	
Empyema	1 (2.3)	0	
Sepsis	1 (2.3)	0	

DM=Diabetes mellitus, IHD=Ischemic heart disease, HLP=Hyperlipidemia, HTN=Hypertension, ICU=Intensive care units

excluded from the drug and control groups, respectively. Finally, 43 patients in the drug group and 39 patients in the control group completed the study [Figure 1].

Table 1 presents the basic demographic and clinical information of the patients in the two groups; no difference was observed between the two groups in terms of these parameters.

Table 2 shows the results of SCr, BUN, and CrCl in patients in the drug and control groups and also their comparison. A significant increase in SCr and BUN and a significant reduction in CrCl were observed in both groups; however, there was no significant difference between the two groups in terms of the three indices at any time. Furthermore, for all three parameters, there was no significant difference between the two groups in terms of the trend of changes.

As shown in Table 3, the changes in the level of urinary NGAL were not significantly different between the two groups.

Table 4 shows the frequencies of AKI occurrence, including its stages, in both groups. As shown, there were 11 cases of AKI (28.2%) in the control group versus 8 cases (18.6%) in the drug group. Moreover, the frequency of stages 2 and 3 AKI was lower in the drug group compared to the control; however, the differences between the two groups were not statistically significant ($P = 0.303$).

Discussion

In this study, it was found that co-administration of NAC with colistin could not inhibit the increase in SCr and BUN, reduction of CrCl, and development of AKI by this antibiotic.

Most studies in the field of reducing colistin nephrotoxicity have been of the animal type. Yousef *et al*. showed the protective effect of melatonin on the nephrotoxicity of colistin in rats, highlighting the potential of using an antioxidant to enhance the therapeutic window of colistin.[9] In the study of Ghlissi *et al*., concomitant use of vitamins E and C with colistin in rats reduced renal tubular damage.[10]

An *in vitro* study on the neuroblastoma-2a cells indicated that NAC inhibited the increase of colistin-dependent apoptosis.[8] Ceylan *et al*. examined the molecular mechanism of colistin-induced nephrotoxicity and found that the expression levels of eNOS, SOD2, and matrix metalloproteinase-3 were significantly higher in rats treated with colistin and NAC compared to those treated with colistin alone, and NAC decreased colistin-induced nephrotoxicity.[7]

Bozkurt *et al*. examined the relationship between consumption of NAC and colistin-induced nephrotoxicity in a retrospective cohort study. A daily dose of colistin 150 mg 2 to 3 times/day was administrated in both groups, and two to three 300 mg intravenous doses

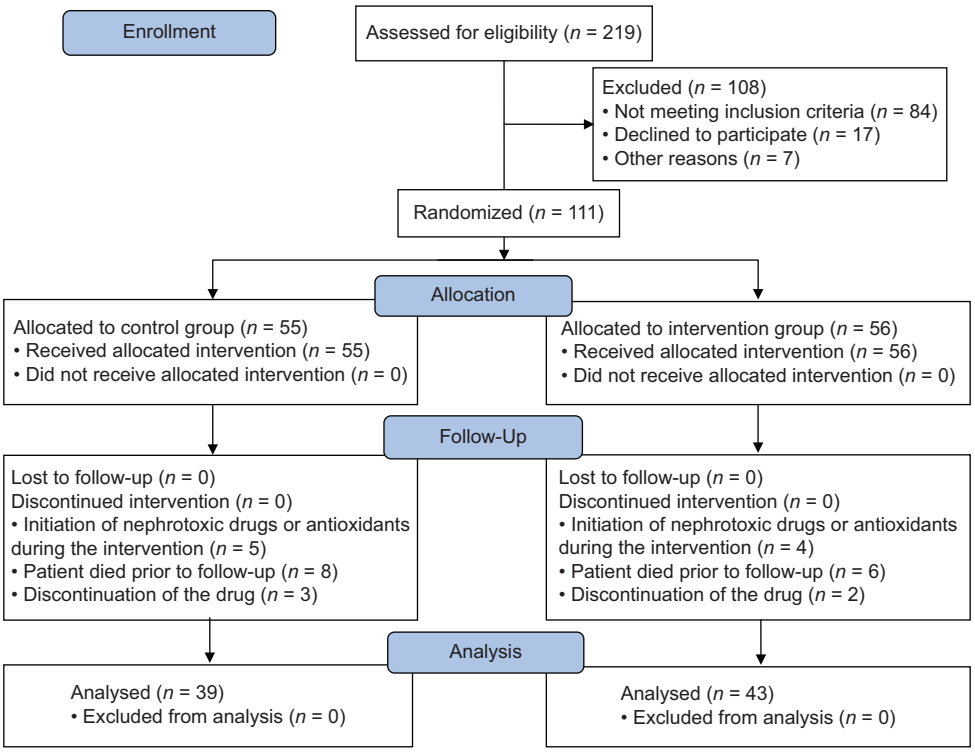

Figure 1: CONSORT flowchart of the study

Table 2: Comparison of values of the parameters between two groups; the values indicate mean±SD (range)

Parameter	Study Stage	Intervention group (*n*=43)	Control group (*n*=39)	*P*[a]
SCr (mg/dL)	Day 0	0.82±0.20 (0.5-1.2)	0.80±0.19 (0.5-1.2)	0.665
	Day 2	0.84±0.21 (0.5-1.5)	0.87±0.36 (0.5-2.7)	0.701
	Day 4	1.14±1.22 (0.5-8.0)	0.95±0.49 (0.5-3.2)	0.362
	Day 6	1.04±0.99 (0.5-6.8)	1.13±1.01 (0.5-5.9)	0.700
	Day 8	1.12±0.98 (0.5-6.2)	1.18±1.00 (0.5-5.8)	0.779
	Day 10	1.14±0.88 (0.5-5)	1.27±1.27 (0.5-6.7)	0.679
P[b]		<0.001	<0.001	0.051
BUN (mg/dL)	Day 0	16.14±6.81 (5-34)	14.82±7.82 (5-39)	0.417
	Day 2	16.71±8.30 (5-39)	15.13±8.00 (6-40)	0.389
	Day 4	18.65±14.88 (5-92)	16.30±8.60 (5-37)	0.399
	Day 6	20.26±21.87 (5-140)	17.30±10.48 (5-52)	0.458
	Day 8	21.28±19.02 (5-110)	19.57±14.30 (5-60)	0.686
	Day 10	22.88±19.00 (8-95)	20.80±16.83 (5-69)	0.681
P[b]		<0.001	<0.001	0.238
CrCl (ml/min)	Day 0	96.12±18.49 (49.43-134.52)	100.17±22.53 (57.68-140.71)	0.375
	Day 2	94.73±19.71 (33.97-134.52)	96.17±28.24 (19.48-155.69)	0.792
	Day 4	89.53±26.03 (20.52-134.52)	92.68±30.70 (15.86-140.71)	0.619
	Day 6	88.67±26.97 (10.48-134.52)	90.37±35.41 (7.57-140.73)	0.923
	Day 8	84.73±30.07 (11.72-134.52)	84.57±36.44 (7.73-138.35)	0.984
	Day 10	82.01±30.50 (15.20-114.45)	84.81±38.88 (6.49-140.73)	0.775
P[b]		<0.001	<0.001	0.240

[a]Independent samples *t*-test, [b]Repeated measures ANOVA. SCr=Serum creatinine level, BUN=Blood urea nitrogen, CrCl=Creatinine clearance, SD=standard deviation

Table 3: Change of urinary NGAL and its comparison between two groups; the values indicate mean±SD

Time	Group		Difference (95% CI)	*P*[a]
	Intervention group	Control group		
Day 0	201.14±215.38	556.94±378.98	335.80±112.01 (−586.73-−124.86)	0.004
Day 5	119.58±48.64	496.38±440.63	376.80±110.98 (−612.76-−140.84)	0.004
Difference (95% CI)	81.56±236.03 (−61.07-224.20)	60.55±329.63 (−115.09-236.21)	21.01±108.94 (−202.52-244.54)	0.849
P[b]	0.237	0.474		

[a]Independent samples *t*-test, [b]Paired samples *t*-test. CI=Confidence interval, NAC=N-acetylcysteine, NGAL=neutrophil gelatinase-associated lipocalin, SD=standard deviation

Table 4: Comparison of the frequency of AKI between two groups

Parameter	Drug (*n*=43), *n* (%)	Control (*n*=39), *n* (%)	*P*[a]
Number of AKI cases	8 (18.6)	11 (28.2)	0.303
AKI stage			
Stage 1	5 (11.6)	4 (10.3)	0.404
Stage 2	1 (2.3)	4 (10.3)	
Stage 3	2 (4.7)	3 (7.7)	

[a]Chi-square test. AKI=Acute kidney injury

of NAC were administrated for the drug group once daily. According to the results, consumption of NAC did not have any positive effect in the prevention of colistin-induced nephrotoxicity, so that the incidence of AKI was similar in both groups.[22] These results are consistent with our observations.

In the present study, since NAC was associated with a further reduction in the level of urine NGAL compared to the control group, an increase in NAC dose and/or the duration of intervention might be associated with a significant effect of the antioxidant in reducing colistin-induced nephrotoxicity. NGAL is a 25-kDa protein with 178 amino acids. In response to nephrotoxic or ischemic stimuli, NGAL is produced in the renal tubules and excreted in the urine. Therefore, urine NGAL can act as a highly sensitive and noninvasive biomarker for the early diagnosis of AKI.[23] It is a reliable diagnostic biomarker and predictor of AKI, and its serum and urine levels increase 48 h before any significant change in SCr and is more sensitive than SCr in patients with AKI due to ischemia or nephrotoxic drugs.[24]

In this study, there were fewer cases of AKI in NAC group compared to the control group. Although the difference was not significant, it could be indicative of the potential of NAC for the reduction of colistin-induced

nephrotoxicity at higher doses. Of note, fewer cases of stages 2 and 3 AKI in NAC group compared to the control also reinforce this possibility.

Our study limitations include its small sample size, lack of histological examination of the kidney, short duration of the intervention, and lack of placebo (consequently, the impossibility of blinding). Nevertheless, this study is considered the first prospective clinical trial for evaluating the effectiveness of NAC to prevent colistin-induced nephrotoxicity; hence, it used a suitable biomarker NGAL for better judgment. Similar placebo-controlled studies with higher sample sizes and higher doses of NAC are recommended to confirm or reject the results.

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Conflicts of interest

There are no conflicts of interest.

Authors' contribution

A. Hakamifard selected the patients based on the inclusion criteria, prescribed the tablets, and evaluated the patients regarding the clinical response. R. Soltani designed the study, analyzed the data, and revised the manuscript. F. Shafiee performed the assays on blood and urine samples. S. Assarzadeh and S. Mosayebi collected the patients' data, monitored them during the intervention, and drafted the manuscript.

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