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Could nephrotoxicity due to colistin be ameliorated with the use of *N*-acetylcysteine?

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Abstract *Purpose:* The protective effect of *N*-acetylcysteine (NAC) on nephrotoxicity due to contrast nephropathy and reperfusion-induced ischemia has been reported in experimental models. However, its efficacy on colistin-induced nephrotoxicity has not been elucidated yet. The primary aim of this study was to evaluate the nephrotoxic effect of colistin and to investigate the possible protective effect of NAC on colistin-induced nephrotoxicity. The secondary aim was to research the systemic effects of nephrotoxicity-induced oxidative stress on the lung. *Methods:* Eighteen female Sprague-Dawley rats were randomly assigned and were given (a) 1 ml/kg sterile saline, (b) 300,000 IU/kg/day colistin, and (c) 300,000 IU/kg/day colistin and 150 mg/kg NAC for six consecutive days. *Results:* Plasma blood urea nitrogen (BUN), creatinine, urinary creatinine, urinary protein, plasma TNF-alpha levels, renal tissue superoxide dismutase

(SOD) and malondialdehyde (MDA) activity and immunocytochemical findings were evaluated. Colistin exerted nephrotoxicity and achieved a significant increase in plasma BUN and creatinine levels and renal tissue SOD levels. NAC exhibited no significant effect on biochemical parameters but reduced renal tissue SOD level and reversed immunocytochemical staining of inducible nitric oxide synthase (i-NOS) and neurotrophin-3. Increased oxidative stress in the lung tissue of the rats treated with colistin has also been documented. Additionally, NAC significantly reduced the immunostaining of endothelial NOS (e-NOS) and i-NOS in the lung tissue. *Conclusions:* Colistin-induced renal toxicity may be attributable to oxidative damage. The combined treatment of colistin plus NAC seems to have a beneficial role in restoration of the oxidant injury which may be related to its antioxidant effect.

Keywords Colistin · Nephrotoxicity · *N*-Acetylcysteine (NAC) · Oxidative stress · i-NOS · NT-3

Introduction

Colistin is a cyclic cationic polypeptide antibiotic which is widely used after the appearance of gram negative bacteria

which are resistant to almost all classes of commercially available antibiotics [1–3]. Nephrotoxicity is the most frequently observed limited side effect and results in either early discontinuation of treatment or worse prognosis [1, 4].

N-Acetylcysteine (NAC) is a thiol-containing compound which plays a key role in detoxification of free radicals [5]. A number of reports indicated the protective effect of NAC on drug-induced nephrotoxicity but the outcome on colistin-induced nephrotoxicity has not been investigated yet [6, 7].

The primary aim of this experimental study was to evaluate the nephrotoxic effect of colistin and to research the possible protective effect of NAC on colistin-induced nephrotoxicity. In order to explore the potential role of oxidative stress in colistin-induced nephrotoxicity, two enzymatic markers which induce nitric oxide (NO) synthesis [endothelial NOS (e-NOS) and inducible NOS (i-NOS)] and neurotrophin-3 (NT-3), which is a well-known marker of tissue injury, were selected for immunocytochemical analysis. Because increased oxidative stress is not restricted locally and may spread by systemic circulation, we planned to investigate the systemic effects of oxidative stress on the lung, which is a main target of oxidant overload, as a secondary aim.

Materials and methods

Drugs

Colistin was obtained from Forest Laboratories (Bexley, UK) and NAC was obtained from Husnu Arsan Pharmaceuticals (Istanbul, Turkey).

Animals

Adult female Sprague-Dawley rats each weighing 225 ± 25 g were obtained from the experimental animals laboratory of our institute and were kept in appropriate conditions. The study protocol was approved by the animal research ethics committee of the institute. All experiments were performed according to the rules of the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996).

Experimental design

The animals were randomly divided into three groups with six rats each as follows: group 1 was the control group which was administered 1 ml/kg intraperitoneal (i.p.) sterile saline; group 2 was assigned to receive 300,000 IU/kg/day i.p. colistin (colistimethate sodium; 1 million IU/vial); and group 3 was treated with 300,000 IU/kg/day colistin and 150 mg/kg NAC i.p. in each single dose for six consecutive days. On the seventh day, 24 h after the last injection, the animals were killed.

Biochemical assay

Plasma levels of BUN, creatinine, and urine levels of protein and creatinine were measured with standard kits and colorimetric methods. Plasma TNF-alpha levels were investigated by using a standard ELISA kit (KRC3011, BioSource, California). Tissue SOD and MDA levels were measured with colorimetric method by using standard kits (from Cayman, USA, and Oxis Research, Foster City, USA, respectively).

Immunohistochemical examination

For immunohistochemical examination, the Zymed Histostain-Plus Broad Spectrum kit was used (Ref/Cat No. 85-9043, Lot 457501A, Zymed, USA). Slides were examined with a Photo-light microscope (DCM4500 Image Analyze System and QWin Programme, DFC280 Plus Camera, Leica, Germany). A semiquantitative scoring system was used to assess the immunolabeling intensity. The HSCORE was calculated by using the following equation: $HSCORE = \sum Pi(i + 1)$, where i is the intensity of labeling with a value of 1, 2, or 3 and Pi is the percentage of labeled epithelial and stromal cells for each intensity, varying from 0 to 100% [8].

Statistical analysis

SPSS for Windows software was used for the statistical analysis of the results (SPSS for Windows; Chicago, IL, USA). Results are presented as mean \pm SD and percentiles. Paired sample t test, one-way ANOVA analysis of variance, and post hoc multiple comparison test (Bonferroni) were performed on the data of the biochemical variables to examine differences between the groups. A difference was considered statistically significant at $p < 0.05$.

Results

Colistin caused an elevation of BUN and plasma creatinine levels and a decrease in creatinine clearance (CICr) ($p < 0.05$), while urine creatinine and protein levels did not alter ($p > 0.05$). NAC treatment did not change these parameters (Table 1).

Plasma TNF-alpha and tissue MDA levels were not changed by colistin or NAC treatment ($p > 0.05$). However, colistin resulted in an increase in tissue SOD levels in kidney and this increase was prevented with NAC use ($p < 0.05$) [Fig. 1 of the electronic supplementary material (ESM)].

Table 1 Effects of colistin alone and its combination with NAC on plasma urine, plasma creatinine, urine creatinine, urine protein, and creatinine clearance levels in rats

	Control (n = 6)	Colistin (n = 6)	Colistin + NAC (n = 6)
Urea (mg/dl)	27.8 ± 4.5	53.6 ± 16.0*	63.1 ± 22.9*
Creatinine (mg/dl)	0.62 ± 0.06	1.05 ± 0.28*	1.03 ± 0.32*
Urine creatinine (mg/dl)	80.8 ± 32.2	72.5 ± 17.1	86.6 ± 13.4
Urine protein (mg/dl)	100.2 ± 80.3	66.4 ± 26.1	88.9 ± 52.2
ClCr (ml/min)	0.93 ± 0.10	0.57 ± 0.26*	0.73 ± 0.26

Data are presented in mean ± SD

ClCr creatinine clearance

* $p < 0.05$ versus control group

Kidney histopathology

Light microscopy findings were comparable among all three groups. Immunohistochemical evaluation of the control group displayed negative to light expression of NT-3 and i-NOS in proximal tubules, distal tubules, and medullary collecting tubules of the kidney. e-NOS staining was prominent in the glomerules and tubular capillary. In the colistin group, high expression of NT-3 and i-NOS was observed in glomerular capillary, distal tubuli, intertubular capillary, and collecting tubules, while e-NOS staining was reduced in these segments ($p < 0.05$). In the colistin + NAC group, the expression of e-NOS was lower in the glomeruli and higher in the distal and collecting tubules. Also, i-NOS activity was decreased in the glomeruli, distal, and collecting tubules. Although immunostaining of NT-3 was clearly weaker than the colistin groups, it was still a bit stronger than the controls (Table 2) (e-NOS results are presented as Fig. 2 of the ESM). i-NOS and NT-3 results are presented in Figs. 1 and 2.

Lung histopathology

Under light microscopy examination, the control group revealed normal histology of the lung and NT-3 expression was found to be moderate to severe. In the colistin group normal alveolar structure was rarely observed. Alveolar saccus and ductus were enlarged and interalveolar septum was thickened. NT-3 immunoreactivity and i-NOS were found to be higher while e-NOS staining was lower than the controls ($p < 0.05$). In the colistin + NAC group, the general characteristics were broadly similar to the control group. The immunoreactivity of e-NOS and i-NOS were lower than in the colistin group, whereas NT-3 staining was comparable (Table 2) (figure not shown).

Table 2 Comparison of the e-NOS, i-NOS, and NT-3 staining scores of the glomerule, distal tubule, collecting tubules, and lung tissue

	Control (n = 6)	Colistin (n = 6)	Colistin + NAC (n = 6)
e-NOS			
Glomerule	119 ± 3.8	96 ± 2.7*	84 ± 3.4* [‡]
Distal tubule	147 ± 3.9	74 ± 2.9*	142 ± 40.5 [‡]
Collecting tubule	126 ± 3.5	76 ± 3.1*	93 ± 3.6* [‡]
Lung	113 ± 2.2	86 ± 2.7*	74 ± 3.5* [‡]
i-NOS			
Glomerule	45 ± 4.6	102 ± 4.7*	96 ± 4.7*
Distal tubule	20 ± 1.4	155 ± 2.7*	150 ± 4.7*
Collecting tubule	63 ± 5.3	194 ± 8*	133 ± 4.5* [‡]
Lung	47 ± 4.2	94 ± 4.3*	86 ± 4.7* [‡]
NT-3			
Glomerule	55 ± 3.4	144 ± 7.6*	143 ± 3.6*
Distal tubule	24 ± 2.9	116 ± 6.3*	106 ± 4.5* [‡]
Collecting tubule	64 ± 3.5	216 ± 6.3*	197 ± 4.5* [‡]
Lung	45 ± 3.9	131 ± 7.9*	132 ± 3*

Data are presented in mean ± SD

* $p < 0.05$ versus control group; [‡] $p < 0.05$ versus colistin group

Discussion

The present study indicated that colistin caused nephrotoxicity in rats and although concomitant use of NAC did not significantly improve biochemical parameters and ClCr, it reversed the increased levels of SOD and improved the immunohistochemical expression of NT-3 and i-NOS which was predominantly observed in the collecting tubules of the colistin group. In addition, increased e-NOS and i-NOS staining, which has also been documented in the lung tissue of the colistin group, was recovered with NAC use.

Colistin is a valuable therapeutic option in clinical practice which is increasingly used for multidrug resistant gram negative pathogens [1–4, 9–12]. Renal toxicity due to colistin is reported to be dose dependent and mainly present as acute tubular necrosis [10]. Although the exact mechanism is still a matter of debate, D-amino acid and fatty acid components are proposed to be the causes of nephrotoxicity due to colistin [10].

NAC is an inexpensive, easy-to-use drug with no significant toxicity. NAC exhibits its antioxidant property in several ways including interaction with the electrophilic groups of reactive oxygen species (ROSs) and being a glutathione (GSH) precursor [13]. The pathogenic role of oxidative stress and the protective effect of NAC have been documented in sepsis, reperfusion-induced ischemia, and drug-induced nephrotoxicity due to gentamicin and cisplatin [14–17]. Nevertheless the possible effect on colistin-induced nephrotoxicity has not been evaluated yet.

The results of the present study are in accordance with the previous reports which verified nephrotoxicity due to

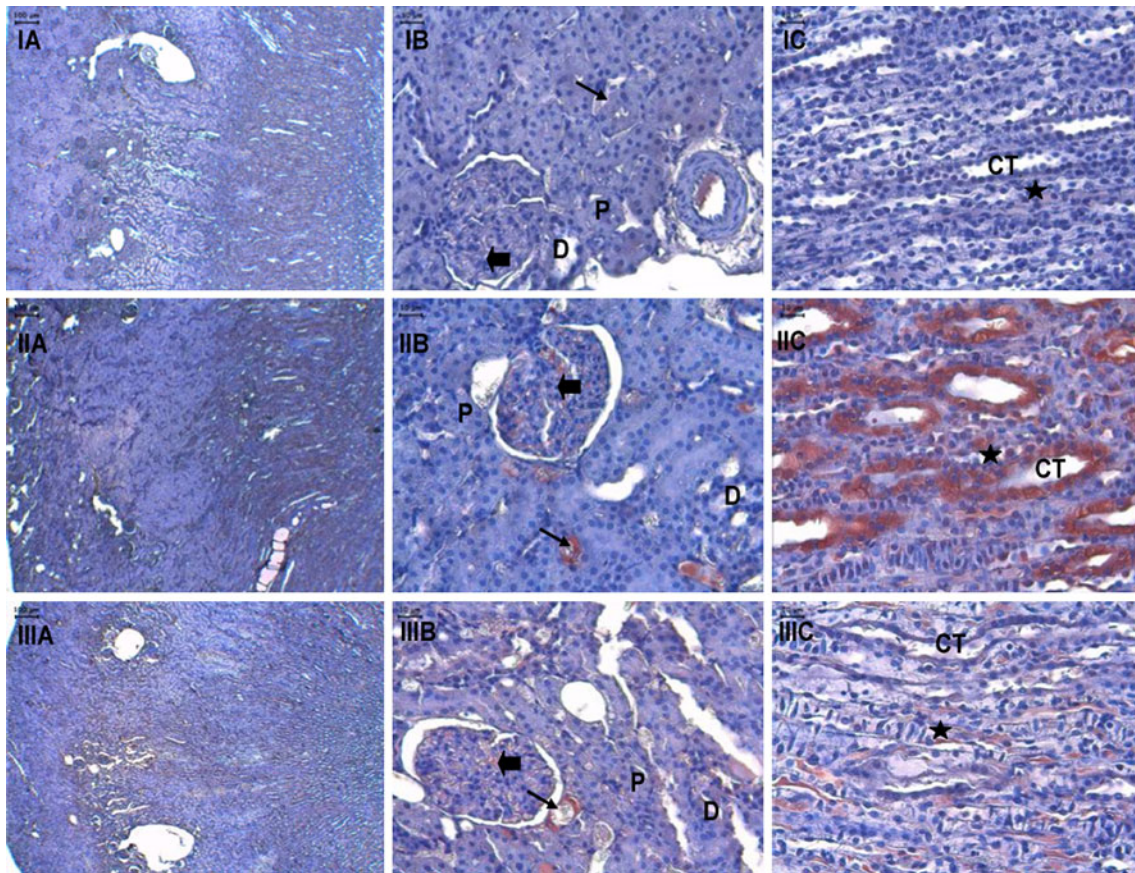


Fig. 1 i-NOS immunocytochemical staining of kidney tissue control group (**I**), colistin group (**II**), colistin + NAC group (**III**). **a** $\times 100$, *thick arrows* glomerular capillary, *thin arrows*

tubular capillary, *P* proximal tubule, *D* distal tubule. **b** $\times 400$, *CT* collecting tubule, *asterisks* capillary between collecting tubules. **c** $\times 400$

colistin use [2, 9]. Although light microscopy revealed comparable results, increased renal function tests indicate acute tubular necrosis in the study group. The cellular sources of NO in the renal tissue are mainly proximal and distal tubules [18]. We have demonstrated the augmentation of e-NOS, i-NOS, and NT-3 expression due to colistin use in the tubular region. In addition, the expression was significantly decreased due to concomitant use of NAC in the tubular zone. The reversed immunocytochemical staining of i-NOS activity, rather than e-NOS may indicate i-NOS as the underlying cause of nephrotoxicity related to colistin-induced oxidative damage. This idea can also be supported with the reversed i-NOS staining at the tubular zone which is the main affected region in colistin-induced nephrotoxicity. Reduction of the elevated SOD levels and reversal of the increase in the tubular immunohistochemical expression of NT-3 and i-NOS with the use of NAC indicate that oxidative stress may be the underlying mechanism of nephrotoxicity due to colistin. NAC may have a beneficial role in restoring the tissue, although not reversing all alterations. More interestingly, the improvement of the immunocytochemical staining of the lung

tissue due to NAC use revealed that the oxidative stress induced by colistin use is not restricted to the kidney. Excessively produced oxidants could migrate to the lung through systemic circulation and result in the demonstrated damage. Systemic effects of oxidant overload due to drug nephrotoxicity should be highlighted with further investigations.

There may be several reasons for the reduced SOD rank and reversed immunocytochemical findings without any alteration in BUN and creatinine levels. At first, the dosage used or the duration of the treatment may be inadequate. Second, NAC was administered simultaneously with colistin in this experiment. Early administration which is suggested for contrast nephrotoxicity may augment the protective effect [7]. Third, the rats were not further hydrated during the experiment which is a widely used therapeutic option to prevent nephrotoxicity.

To our knowledge, this is the first study which investigates the protective effect of NAC on colistin-induced nephrotoxicity. Colistin-induced renal toxicity may be attributable to oxidative damage, and administration of NAC seems to have a beneficial role in restoring kidney

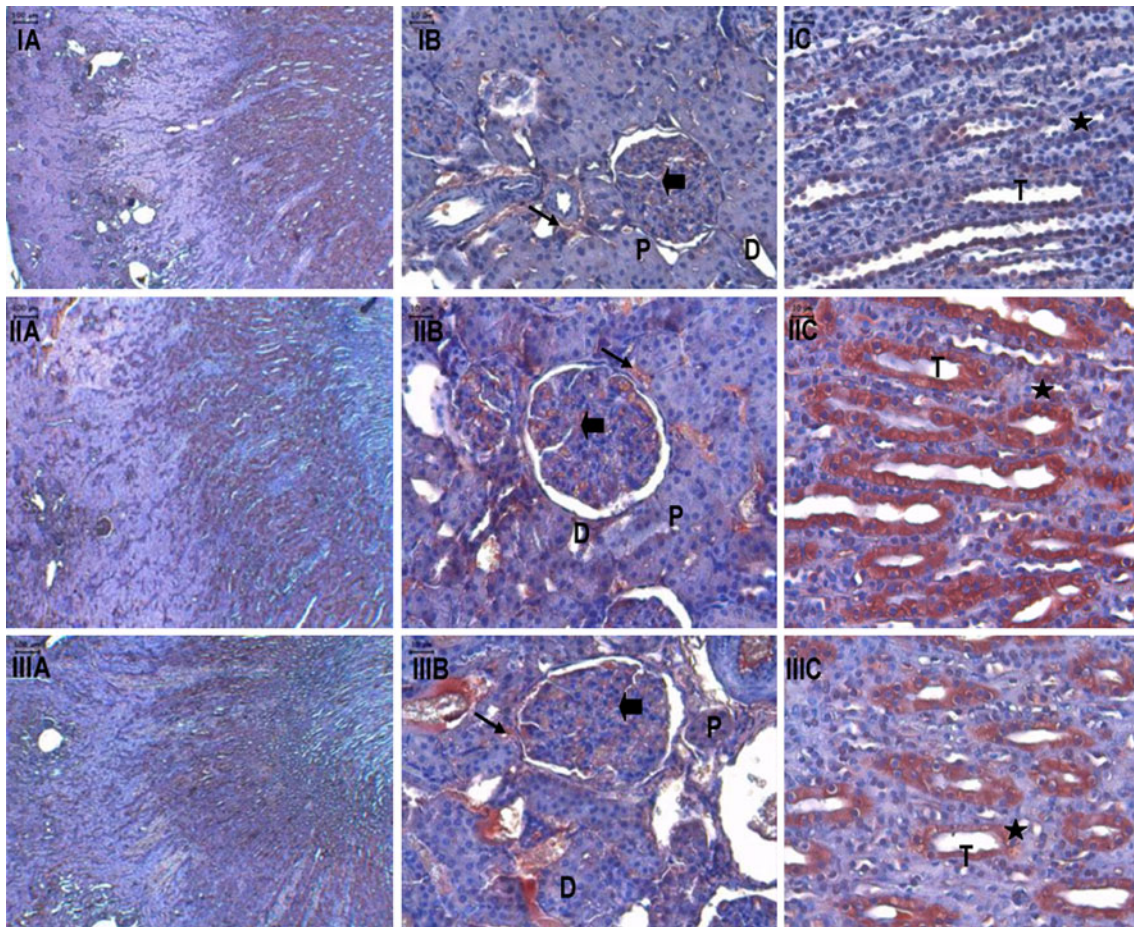


Fig. 2 NT-3 immunocytochemical staining of kidney tissue control group (I), colistin group (II), colistin + NAC group (III). **a** $\times 100$, thick arrows glomerular capillary, thin arrows tubular capillary,

P proximal tubule, *D* distal tubule. **b** $\times 400$, *CT* collecting tubule, asterisks capillary between collecting tubules. **c** $\times 400$

injury. Further research is warranted to enlighten us about the possible effect, appropriate dose, and duration of the NAC therapy on colistin-induced nephrotoxicity.

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