Ascorbic acid protects against the nephrotoxicity and apoptosis caused by colistin and affects its pharmacokinetics

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Objectives: The use of colistin in the treatment of life-threatening Gram-negative infections is associated with a high rate of nephrotoxicity that is dose limiting. This study aimed to examine the nephroprotective effect of ascorbic acid against colistin-induced nephrotoxicity.

Methods: Rats were treated intravenously twice daily with saline, colistin (cumulative dose of 36.5 mg/kg), a combination of ascorbic acid (50 or 200 mg/kg) and colistin, or ascorbic acid (200 mg/kg) over 7 days. Colistin-induced apoptosis was examined in rats over 5 days and in vitro using rat renal proximal tubular cells NRK-52E over 24 h with and without ascorbic acid. The effect of co-administered ascorbic acid on colistin pharmacokinetics was investigated.

Results: The 24 h urinary excretion of N-acetyl-β-D-glucosaminidase, a sensitive marker for tubular damage, was significantly lower (P<0.0001) in the colistin/ascorbic acid 200 mg/kg group. Significant histological abnormalities (P<0.01) were detected only in the kidneys of the colistin group, which also had the highest percentage (30.6±7.8%) of apoptotic cells (P<0.005). In the cell culture studies, the percentage of apoptotic cells was significantly higher in the presence of 0.1 mM colistin alone (51.8±2.0%; P<0.0001) than in the presence of ascorbic acid, which decreased the apoptotic effect in a concentration-dependent manner. Ascorbic acid (200 mg/kg) altered colistin pharmacokinetics, as the total body clearance decreased from 3.78±0.36 mL/min/kg (colistin group) to 2.46±0.57 mL/min/kg (P=0.0024).

Conclusions: This is the first study demonstrating the protective effect of ascorbic acid against colistin-induced nephrotoxicity and tubular apoptosis. Co-administration of ascorbic acid has the potential to increase the therapeutic index of colistin.

Keywords: polymyxin E, vitamin C, rat kidney tubular cells

Introduction

Colistin (also known as polymyxin E) is one of the few remaining therapeutic options available to treat life-threatening infections caused by multidrug-resistant (MDR) Gram-negative bacteria, in particular Pseudomonas aeruginosa, Acinetobacter baumannii and Klebsiella pneumoniae.1–5 Clinically, colistin is administered parenterally as sodium colistin methanesulphonate (CMS), an inactive pro-drug that is converted into colistin, the antibacterial and toxic entity.4–6 The early use of CMS/colistin was linked to high rates of toxicity, especially nephrotoxicity, and therefore its use waned7–10 until its recent resurgence due to increasing resistance in Gram-negative bacteria.5 The reported nephrotoxicity rates in patients receiving currently recommended dosage regimens are up to 45%–55%,11–16 even though a recent clinical population pharmacokinetic study demonstrated these dosage regimens are suboptimal in many patients.16 This situation is destined to deteriorate with diminishing bacterial susceptibility to colistin.17,18 Thus nephrotoxicity is the major adverse effect that impedes the ability to administer higher doses of CMS.

Recent studies in rats suggest that oxidative stress has a role in colistin-induced nephrotoxicity.19,20 Oxidative stress has been suggested to play a key role in the nephrotoxicity caused by many other drugs, including gentamicin, vancomycin and cisplatin; reactive oxygen species generated via mitochondria have been shown to initiate renal cell apoptosis, ultimately leading to renal dysfunction.21 Ascorbic acid, a chain-breaking antioxidant and free radical scavenger,22–24 has a protective effect against nephrotoxicity caused by gentamicin, vancomycin...
Ascorbic acid attenuates colistin nephrotoxicity

and cisplatin in animals. Considering the common use of ascorbic acid in patients, our study was aimed at examining whether ascorbic acid protects against colistin-induced nephrotoxicity and apoptosis and its possible effect on the pharmacokinetics of colistin.

Materials and methods

Chemicals and reagents

Colistin sulphate (EPS grade) was purchased from Zhejiang Shenghua Biology Co., Ltd (Zhejiang, China). A stock solution (10 mg/mL colistin base) was prepared in sterile normal saline and stored at 4 °C. Rat kidney proximal tubular cells (NRK-52E) were from ATCC (VA, USA). Ascorbic acid, Trichloroacetic acid, 9-Fluorenylmethyl chloroformate (FMOC-Cl), p-nitrophenol, p-nitrophenyl N-acetyl-β-D-glucosaminide, Dulbecco’s modified Eagle’s medium, PBS and paraformaldehyde (PFA) were purchased from Sigma–Aldrich (New South Wales, Australia). Fetal bovine serum (FBS) was from Invitrogen Australia Pty Ltd (Victoria, Australia). All other chemicals were of analytical grade.

Animals

The Monash Institute of Pharmaceutical Sciences Animal Ethics Committee (Monash University) approved this study. Experiments were performed in male Sprague-Dawley rats (mean ± SD body weight 272±10 g; Monash Animal Services). Rats were housed individually in metabolic cages in a temperature- and humidity-controlled facility with a 12 h light–dark cycle and were given free access to food and water. After 2 days of acclimation, rats had a cannula inserted in each of the jugular vein and carotid artery.

Effect of ascorbic acid on colistin-induced nephrotoxicity and colistin pharmacokinetics in rats

Reproducible colistin-induced nephrotoxicity was established by administration of increasing doses of colistin twice daily for 7 days (a cumulative dose of 36.5 mg/kg), as described previously. Biochemical evaluation (plasma creatinine and urinary N-acetyl-β-D-glucosaminidase (NAG) excretion) and histological examination were conducted (see below). The nature and extent of kidney damage caused, including the likelihood of its reversibility, were considered representative of colistin-induced nephrotoxicity in patients.

Rats were divided into five groups (n=5 each) and dosed via the jugular vein twice daily for 7 days with: (i) saline (control group); (ii) colistin as described above (colistin group); (iii) ascorbic acid 200 mg/kg (ascorbic acid 200 group); (iv) ascorbic acid 200 mg/kg 20 min prior to each colistin dose (colistin/ascorbic acid 200 group); and (v) ascorbic acid 50 mg/kg 20 min prior to each colistin dose (colistin/ascorbic acid 50 group). The twice-daily doses of saline, colistin and ascorbic acid, as relevant, were administered, with the second dose being administered 8 h after the first dose on each day. Urine was collected in 24 h intervals 3 days prior to commencing the treatments (baseline), for 6 h after administration of the first dose of colistin on day 1 and in 24 h intervals on days 1, 3, 5 and 6 thereafter; a chilling chamber at 4 °C was employed to ensure the stability of NAG. The volume of urine was measured and aliquots were stored at −80 °C until analysis for NAG excretion and colistin. Samples of blood (~0.3 mL) were collected via the carotid artery immediately before the treatments, at 30 min after the morning dose on days 3 and 6 and at the time the rats were sacrificed on day 7. To assess the pharmacokinetics of colistin, blood (~0.3 mL) was collected from rats in the colistin and the colistin/ascorbic acid 200 mg/kg groups at 10, 30, 60, 90, 120, 180 and 360 min on day 1 after the first dose of colistin (0.5 mg/kg). Plasma samples were stored at −80 °C pending quantification of colistin; plasma creatinine was measured at baseline and on day 6. On day 7, rats were sacrificed 3 h after the last dose and the kidneys were removed. The left kidneys were stored at −80 °C for measurement of colistin and superoxide dismutase (SOD) and the right kidneys were fixed in 10% neutral buffered formalin for histological examination.

Histological examination

For light microscopic examination, the formalin-fixed kidneys were dehydrated in an ascending ethanol series (70%, 90% and 100%), cleared in xylene and embedded in paraffin wax. Sections (5 μm) were stained with haematoxylin and eosin. The samples were coded and examined by a pathologist (P. A. H.) who was blinded to the treatment groups. Lesions were categorized into three grades as previously described: grade 1, mild acute tubular damage with tubular dilation, prominent nuclei and a few pale tubular casts; grade 2, severe acute tubular damage with necrosis of tubular epithelial cells and numerous tubular casts; and grade 3, acute cortical necrosis/infarction of tubules and glomeruli with or without papillary necrosis. The grades were given the following scores: grade 1 = 1, grade 2 = 4 and grade 3 = 10. The percentages of the kidney slices affected were assigned the following scores: <1% = 0, 1% to <5% = 1, 5% to <10% = 2, 10% to <20% = 3, 20% to <30% = 4, 30% to <40% = 5 and ≥40% = 6. The overall score was calculated as the product of percentage score and grade score. Finally, a semi-quantitative score (SQS) for renal histological changes was assigned as follows: SQS 0=no significant change (overall score <1), SQS 1=mild damage (overall score 1 to <15), SQS 2=mild to moderate damage (overall score 15 to <30), SQS 3=moderate damage (overall score 30 to <45), SQS 4=moderate to severe damage (overall score 45 to <60) and SQS 5=severe damage (overall score 60).

Quantification of biochemical markers and colistin

Plasma creatinine was quantified using a commercial kit (Bioassay Systems, CA, USA); the accuracy and reproducibility (coefficient of variation (CVI)) were 99% and 9%, respectively. Concentrations of NAG in urine were measured; the accuracy and reproducibility were 96% and 5%, respectively. Cu/Zn-SOD activity in kidney homogenate was assessed by measuring the dismutation of superoxide radicals generated by xanthine oxidase and hypoxanthine (Cayman Chemical, Ann Arbor, MI, USA); the accuracy and reproducibility were 91% and 10%, respectively. Concentrations of colistin in plasma, urine and kidney homogenate were determined by HPLC. Calibration curves in all matrices were constructed with colistin base concentrations ranging from 0.125 to 16.0 μg/mL. Samples with concentrations over the calibration range were reanalysed after appropriate dilutions. For quality control samples containing 0.50 μg/mL and 4.00 μg/mL, the corresponding accuracy and reproducibility were 0.50±0.03 μg/mL and 3.86±0.23 μg/mL for plasma, 0.54±0.02 μg/mL and 3.83±0.15 μg/mL for urine and 0.47±0.07 μg/mL and 3.90±0.13 μg/mL for kidney homogenate.

Effect of ascorbic acid on colistin-induced apoptosis in rats

Our preliminary study showed apoptosis in rat kidney cells within 5 days of initiating colistin treatment. Therefore the 5 day colistin regimen employed in this apoptosis study was the same as the first 5 days in the 7 day regimen described above. Rats were divided into four groups (n=3 each) and dosed via the jugular vein twice daily for 5 days with: (i) saline (control group); (ii) increasing doses of colistin (0.5 and 1.0 mg/kg (day 1), 1.25 and 1.25 mg/kg (day 2), 1.75 and 2.75 mg/kg (day 3), 4.0 mg/kg twice daily (day 4) and 4.0 mg/kg...
(day 5); cumulative dose was 20.5 mg/kg (colistin group); (iii) ascorbic acid 200 mg/kg (ascorbic acid group); and (iv) ascorbic acid 200 mg/kg 20 min prior to each colistin dose (colistin/ascorbic acid group). The kidneys were fixed in 4% PFA for apoptosis analysis and histological examination (see above). Apoptosis was assessed using the immunohistochemical TUNEL procedure based on in situ 3′OH-end tailing of double-strand breaks in DNA of apoptotic cells (TUNEL Universal Apoptosis Detection kit; GenScript, Piscataway, NJ, USA). Images were obtained at the Monash Micro-Imaging Facility.

**Effect of ascorbic acid on colistin-induced apoptosis in cell culture**

Rat proximal tubular cells (NRK-52E) were cultured using Dulbecco’s modified Eagle’s medium supplemented with 10% FBS. For experimental use, these cells were plated onto a Nunc Lab-Tek Chamber Slide system in a medium containing 0.1% FBS and incubated at 37°C in a humidified atmosphere of 5% CO₂ for 3 h. Subsequently, cells were treated with or without colistin (0.1 mM) in the presence and absence of ascorbic acid (0.01, 0.1, 1.0 and 10 mM) for 24 h. At the end of treatment cells were washed with PBS, fixed with 4% PFA and stored at −80°C until measurement of apoptosis (see above).

**Data analyses**

Colistin pharmacokinetics in plasma were analysed using non-compartmental module 201 in WinNonlin (version 5.2; Pharsight Corp., Cary, NC, USA). Prior to statistical comparisons, all continuous variables were tested for normal distribution and homogeneity of variance among groups using the Shapiro–Wilk test and Levene’s test, respectively. An unpaired t-test was used to compare the pharmacokinetic parameters between the colistin and colistin/ascorbic acid 200 mg/kg groups. For plasma creatinine, a paired t-test was used, and for SOD activity, the groups were compared using one-way analysis of variance (ANOVA). Repeated measures ANOVA was used for urinary NAG excretion. If significant differences were found (P < 0.05), the treatment groups were compared using Tukey’s test. For the histological scores and the percentage of apoptotic cells, Kruskal–Wallis one-way ANOVA by ranks was conducted. A P value < 0.05 was considered significant.

**Results**

In the rats treated with colistin for 7 days (cumulative dose 36.5 mg/kg), the 24 h urinary NAG excretion was significantly increased (P < 0.0001) on days 1, 3, 5 and 6 relative to baseline, and on those respective days was significantly higher (P < 0.0001) than for the control, ascorbic acid 200 mg/kg and colistin/ascorbic acid 200 mg/kg groups (Figure 1). For the colistin/ascorbic acid 50 mg/kg group, NAG was significantly higher (P < 0.01) than for the baseline and the control group only on day 6. Plasma creatinine increased significantly significantly in the colistin (P < 0.01) and colistin/ascorbic acid 50 mg/kg (P < 0.05) groups between baseline (0.33 ±0.04 and 0.44 ±0.1 mg/dL, respectively) and day 6 (0.60 ±0.08 and 0.56 ±0.08 mg/dL, respectively), but not in the control and colistin/ascorbic acid 200 mg/kg groups. In the kidneys of the colistin group, significant histological abnormalities (P < 0.01) were observed, but not in the other groups (Figure 2 and Table 1). For SOD activity, there was no significant difference among the groups in the kidneys on day 7 (P > 0.05, data not shown).

Having observed colistin-induced histological and biochemical abnormalities, and amelioration by ascorbic acid over 7 days, further studies were conducted in rats treated for 5 days in order to determine whether apoptotic effects occurred. The morphological appearance of the kidney tissue sections of those rats was comparable to the other groups when stained with haematoxylin and eosin. Renal tissue obtained from the colistin group showed a significantly higher (P < 0.0001) percentage of proximal tubule epithelial cells with positive TUNEL staining of the nuclei compared with renal tissues obtained from the control, colistin/ascorbic acid 200 mg/kg and ascorbic acid 200 mg/kg groups (Figure 3). The percentage of apoptotic cells in rat kidney tissue from the colistin group (30.6 ± 7.8%) was significantly higher (P < 0.005) than for the control (4.2 ± 0.5%), ascorbic acid 200 mg/kg (4.5 ± 0.6%), and colistin/ascorbic acid 200 mg/kg (10.0 ± 0.6%) groups. In the cell culture experiments, the percentages of apoptotic cells were significantly lower in the presence of ascorbic acid at molar concentrations greater than or equal to that of colistin (P < 0.0001; Figure 4). However, complete protection against colistin-induced apoptosis was not observed, but there was a significant protective effect of ascorbic acid 200 mg/kg.

**Figure 1.** Mean (± SD) urinary excretion of NAG in the control, colistin, ascorbic acid 200 mg/kg, colistin/ascorbic acid 50 mg/kg and colistin/ascorbic acid 200 mg/kg groups. *Significantly different from the baseline for the same group and control group on the same day (P < 0.0001).
apoptosis was observed only in the presence of ascorbic acid at molar concentrations ≥10-fold that of colistin (Figure 4).

The mean plasma colistin concentration–time profiles after the first intravenous dose of colistin (0.5 mg/kg) either alone or following the administration of ascorbic acid (200 mg/kg) are shown in Figure 5. Co-administration of ascorbic acid significantly changed the pharmacokinetics of colistin (Table 2). The amount of colistin in urine voided across the 0–6 h interval after the first dose for the colistin group was 0.50 ± 0.19 mg, whereas it was below the limit of quantification (i.e. <0.125 µg/mL) for the colistin/ascorbic acid groups. On days 1, 3 and 5 the amount of unchanged colistin excreted in urine over 24 h was not significantly different between the colistin and colistin/ascorbic acid 50 mg/kg groups; however, it was significantly different between the two groups on day 6. For the colistin and colistin/ascorbic acid 200 mg/kg groups, it was significantly different on days 3, 5 and 6 (P < 0.05; Figure 6). The concentration of colistin in kidney tissue on day 7 was not significantly different (P > 0.05) among the colistin (119.1 ± 40.3 µg/g), colistin/ascorbic acid 50 mg/kg (112.8 ± 12.8 µg/g) and colistin/ascorbic acid 200 mg/kg (92.3 ± 15.4 µg/g) groups.

Discussion

There is an urgent need to optimize the currently recommended dosage regimens of CMS/colistin as a result of increasing reports of resistance to this last-line therapy for Gram-negative ‘superbugs’. Recent pharmacokinetic/pharmacodynamic

Table 1. Histological results for rats treated for 7 days

<table>
<thead>
<tr>
<th>Abnormality grade</th>
<th>control</th>
<th>colistin 200 mg/kg</th>
<th>colistin/ascorbic acid 50 mg/kg</th>
<th>colistin/ascorbic acid 200 mg/kg</th>
</tr>
</thead>
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<td>1</td>
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<td>0, 0, 0, 0</td>
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<td>0, 0, 0, 0</td>
<td>0, 0, 0, 0</td>
<td>0, 0, 0, 0</td>
</tr>
<tr>
<td>SQS for individual rats</td>
<td>0, 0, 0, 0, 0</td>
<td>+1, +3, +4, +1, +4</td>
<td>0, 0, 0, 0</td>
<td>0, 0, 0, 0</td>
</tr>
</tbody>
</table>

Grade 1: mild acute tubular damage with tubular dilation, prominent nuclei and a few pale tubular casts. Grade 2: severe acute tubular damage with necrosis of tubular epithelial cells and numerous tubular casts (acute tubular necrosis).

Colistin/ascorbic acid 50 mg/kg and colistin/ascorbic acid 200 mg/kg: colistin cumulative dose of 36.5 mg/kg and ascorbic acid 50 mg/kg or 200 mg/kg twice daily, respectively.

An SQS was used to grade lesion severity as follows: 0, +1, +2, +3, +4 and +5 correspond to no change, mild, mild to moderate, moderate, moderate to severe, and severe, respectively.

Figure 2. Representative histological images of: (a) kidney of a control rat treated with saline for 7 days showing normal renal cortex and glomeruli (G); (b–d) range of changes in kidneys of rats administered colistin alone [(b) mild tubular damage with tubular casts (★), (c) acute tubular necrosis with preservation of glomeruli (G) and (d) acute cortical necrosis with infarction of tubules and glomeruli (★)]; (e) kidney of a rat treated with ascorbic acid 200 mg/kg alone showing mild focal tubular damage (★); and (f) kidney of a rat in the colistin/ascorbic acid 200 mg/kg group showing normal renal cortex (C) and medulla (M). No significant kidney damage seen in (a), (e) and (f). Original magnification was ×100 for (a–e) and ×20 for (f). This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.
data in critically ill patients indicate that higher doses are required; however, the potential for nephrotoxicity is a major dose-limiting factor. As our preliminary results showed that ascorbic acid (at 10 mM) did not affect the antibacterial activity (i.e. MICs) of colistin, amelioration of the potential for nephrotoxicity would serve to widen the therapeutic window and allow administration of higher daily doses for increased exposure to colistin. Ascorbic acid is an important antioxidant used clinically and has been shown to attenuate the nephrotoxicity caused by other drugs. However, the nephroprotective effect of ascorbic acid against those drugs has not been examined clinically. Our study is the first to demonstrate the protective effect of ascorbic acid against colistin-induced renal tubular apoptosis and nephrotoxicity.

Colistin, rather than CMS, was used in the present study because it is the toxic entity. The dosage regimen employed for colistin generated reproducible kidney damage, in keeping with previous observations. Ascending colistin daily doses were employed to avoid early signs of apparent neurotoxicity after higher initial doses. For ascorbic acid, two different dosage regimens were examined. The high dose of ascorbic acid (200 mg/kg twice a day) was based upon previous reports of its nephroprotective effect against other nephrotoxic drugs, such as cisplatin. After considering animal scaling, this dose is approximately equivalent to the upper limit of the doses for humans (i.e. 2 g/day for a 60 kg adult) by the Food and Nutrition Board. We also examined the nephroprotective effect of a low dose of ascorbic acid (50 mg/kg twice a day). Ascorbic acid was administered 20 min prior to colistin to ensure that circulating plasma concentrations of ascorbic acid were near maximal at the time of colistin administration and also to minimize any possible interaction during administration of the two drugs. The effect of hydration status on colistin-induced nephrotoxicity was not examined in this study.

Histological results showed the nephroprotective effects of both the low and high ascorbic acid dosage regimens (Table 1). All rats in the colistin group showed severe acute tubular damage with necrosis of tubular epithelial cells and numerous tubular casts. One rat in the ascorbic acid group showed mild focal tubular changes only. The histological results were confirmed by monitoring urinary excretion of NAG, an early and sensitive marker for proximal tubular cell damage. Colistin-induced nephrotoxicity was ameliorated in both the colistin/ascorbic acid 50 mg/kg and 200 mg/kg groups on days 1–5, although there was a very modest increase in NAG excretion on day 6 in the colistin/ascorbic acid 50 mg/kg group (Figure 1). The latter is consistent with the small increase

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**Figure 3.** Immunohistochemical images of apoptotic nuclei in sections of kidneys of the rats treated for 5 days with (a) saline, (b) colistin (cumulative dose of 20.5 mg/kg), (c) colistin and ascorbic acid 200 mg/kg and (d) ascorbic acid 200 mg/kg. Original magnification ×100.

**Figure 4.** Percentage of TUNEL-positive apoptotic cells in rat proximal tubular cell line treated with 0.1 mM colistin alone or in the presence of various concentrations of ascorbic acid. *P < 0.005 compared with corresponding control. #P < 0.0001 compared with colistin alone.
(~25%) in plasma creatinine concentration on day 6 relative to the corresponding baseline.

To further examine colistin-induced nephrotoxicity, apoptosis was investigated both in vivo and in vitro. After 5 day treatments, the morphological appearance of the rat kidney tissue sections did not differ across all groups, suggesting that the histological manifestations in the colistin-treated animals observed on day 7 (Figure 2) occurred after day 5. This is in keeping with the time- and cumulative dose-dependent nature of colistin-induced nephrotoxicity. While there was an absence of detectable necrosis across all groups on day 5, apoptosis in renal proximal tubular cells was observed in rats treated with colistin alone, but not in the other groups. Thus, importantly, we have demonstrated that co-administration of ascorbic acid 200 mg/kg protected against both apoptosis on day 5 and histological abnormalities on day 7. This suggests a possible association between colistin-induced apoptosis and nephrotoxicity. In our in vitro cell culture studies, the colistin concentration employed (0.1 mM) was similar to that observed in the kidney tissue (~100 µg/g) at the end of 7 day treatment. It was demonstrated that ascorbic acid inhibited colistin-induced apoptosis in a concentration-dependent manner.

In this study we observed alteration of the pharmacokinetics of colistin by ascorbic acid (Figure 5). Co-administration of 400 mg/kg/day ascorbic acid significantly decreased the total body clearance of colistin and increased its volume of distribution and terminal half-life (Table 2). We have previously suggested that co-administration of another antioxidant, melatonin, protects colistin from degradation by free radicals generated in metabolic processes, thereby decreasing the total body clearance of this non-renal cleared antibiotic. A similar mechanism may be occurring with ascorbic acid in the current study. After multiple administration of colistin, there was a substantial increase in the urinary recovery of unchanged drug on day 6; this increased recovery was most likely due to the decreased functionality of an as yet unidentified transport system(s) involved in the known very extensive tubular reabsorption of colistin. Co-administration of ascorbic acid significantly decreased the urinary recovery of colistin on day 6 compared with the colistin group (Figure 6), consistent with the nephroprotection by ascorbic acid shown in the histological, biochemical and apoptosis results discussed above. The differences in the systemic and renal disposition of colistin cannot be attributed to alteration in the plasma unbound fraction, as this was not altered in the presence of ascorbic acid (P>0.30; data not shown). The mechanism whereby ascorbic acid modifies the

Table 2. Colistin pharmacokinetics (mean ± SD) in rats after administration of 0.5 mg/kg colistin with and without co-administration of 200 mg/kg ascorbic acid (n=5 per group)

<table>
<thead>
<tr>
<th>Group</th>
<th>Clearance (mL/min/kg)</th>
<th>Volume of distribution (mL/kg)</th>
<th>Half-life (h)</th>
</tr>
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<tr>
<td>Colistin</td>
<td>3.78 ± 0.36</td>
<td>369 ± 80</td>
<td>1.20 ± 0.23</td>
</tr>
<tr>
<td>Colistin/ascorbic acid 200</td>
<td>2.46 ± 0.57</td>
<td>703 ± 102</td>
<td>3.91 ± 0.42</td>
</tr>
<tr>
<td>P value</td>
<td>0.0024</td>
<td>0.0004</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Figure 5. Plasma concentration (mean±SD) of colistin versus time profiles after the first intravenous dose of colistin (0.5 mg/kg) either alone (open triangles) or preceded by 200 mg/kg ascorbic acid (filled squares) (n=5 per group). In all rats of the colistin group the plasma colistin concentrations at 360 min were below the limit of quantification of the assay (0.125 µg/mL).

Figure 6. Amount of unchanged colistin excreted in urine on days 1, 3, 5 and 6 following multiple intravenous doses of colistin for 7 days or colistin preceded by ascorbic acid 50 mg/kg or 200 mg/kg. *P<0.05 as compared with the colistin group.
pharmacokinetics, including renal handling, of colistin is under further investigation in our laboratory.

The detailed mechanism of amelioration of colistin-induced nephrotoxicity by ascorbic acid is unknown. However, it is noteworthy that the renal disposition of both colistin and ascorbic acid involves trafficking of each through renal tubular cells. We previously demonstrated both in vivo and ex vivo that colistin undergoes very extensive tubular reabsorption to an extent greater than occurs for water, indicating involvement of carrier-mediated transport. Ascorbic acid also undergoes extensive tubular reabsorption by a saturable active transport process in the proximal tubule. Even though it is possible that ascorbic acid may have inhibited the renal tubular reabsorption of colistin, serving to decrease the tubular cell trafficking of the latter, we believe this is unlikely for two reasons. First, the kidney tissue concentrations of colistin at the end of the treatments were not significantly different among the colistin, colistin/ascorbic acid 50 mg/kg and colistin/ascorbic acid 200 mg/kg groups. Second, inhibition of colistin tubular reabsorption would lead to higher urinary recovery with co-administration of ascorbic acid, but the converse occurred. We believe it is most likely that the nephroprotection was mediated by the free radical scavenging property of ascorbic acid.

In summary, this study is the first to show that ascorbic acid protects against colistin-induced nephrotoxicity in rats and apoptosis of renal tubular cells both in vitro and in vivo. Our finding of the protective effect of ascorbic acid supports the role of reactive oxygen species in colistin-induced apoptosis and nephrotoxicity. The present study reveals the potential for increasing the therapeutic index of this important last-line antibiotic by co-administering ascorbic acid. Clinical studies are warranted to examine the nephroprotective effect of ascorbic acid in patients.

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Transparency declarations
None to declare.

Disclaimer
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