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Evaluation of Tubular Regeneration Following Discontinuation of Colistin in Rat

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ABSTRACT

The study aimed to investigate the spontaneous renal regeneration after stopping colistin methanesulfonate (CMS) which induces tubular damage in rats and the effect of vitamin E (vit E). Animals were given sterile saline (n=6), 300 000 IU/kg/day of CMS (n=24) or 450 000 IU/kg/day of CMS (n=24) for 7 days. Each CMS group was subdivided into 4 subgroups (n=6) and sacrificed as follows: (1) 12 h after stopping CMS, (2) two weeks after stopping CMS, (3) two weeks after stopping treatment with vit E and (4) two weeks after stopping treatment with olive oil (OO). Afterward, plasma creatinine (pCr), urine N-acetyl-b-D-glucosaminidase (uNAG), renal tissue level of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione reductase (GSH), and renal histology were performed. CMS induced tubular damage, increased the NAG and MDA levels and decreased the SOD and GSH levels. After 2 weeks of stopping CMS, there was no significant renal recovery. However, the treatment with vit E improved tubular regeneration and reduced the biochemical damage. Two weeks might not be long enough for significant spontaneous renal regeneration. Renal recovery improvement of vit E could be explained by the reduction of oxidative stress damage.

INTRODUCTION

Colistin is an old class of polypeptide cationic antibiotic which is widely used after the appearance of gram negative bacteria, resistant to almost classes of commercially available antibiotics [1, 2]. Nephrotoxicity is the most frequently observed limited side effect and results in either early discontinuation of treatment or worse prognosis [1-3]. The mechanism of colistin nephrotoxicity still remains unknown; nonetheless it has been reported to be related to total dose of colistin and duration of therapy [4]. Tubular damage due to colistin has been suggested to be reversible upon cessation of therapy [5, 6]. Nevertheless, in experimental setting, renal reversibility after stopping colistin hasn't investigated yet. In patient, it was rarely mentioned and that by the simple normalization of pCr [7, 8].

Antioxidants are well known in offering therapeutic opportunities [9-11]. Vit E, a lipid soluble compound, is a well known antioxidant. Several reports indicated the promising effect of vit E on drug-induced nephrotoxicity such as gentamicin, vancomycin and cisplatin induced nephrotoxicity [12-14].

Therefore we aimed in the present study to investigate the spontaneous renal reversibility after stopping colistin and the effect of treatment with vit E on rats.

MATERIAL AND METHODS

Chemical products

Clinically, colistin is administered parenterally as sodium colistin methanesulfonate (CMS), an inactive pro-drug that is converted into colistin, the antibacterial and toxic entity [15, 16]. CMS was obtained from Aventis-France (1 million IU/vial). Vit E (α-tocopherol acetate) was purchased from Sigma (St. Louis, MO, USA). N-acetyl-β-D-glucosaminidase (NAG) was purchased from Roche (Roche applied science, 68298 Mannheim Germany). Glutathione, nicotinamide adenine dinucleotide phosphate reduced form (NADPH), 5-5'-dithio-bis-2-nitrobenzoic acid (DTNB) and thiobarbituric acid (TBA) were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade and were purchased from standard commercial suppliers.

Animals

Male *wistar* rats weighing 250 ± 20 g were purchased from the breeding centre of the Central Pharmacy (SIPHAT). All animal procedures were conducted in strict conformity with the

local Institute Ethical Committee Guidelines for the care and use of laboratory animals of our institution: they were kept in an environmentally controlled breeding room (temperature: 22 ± 2°C, humidity: 60 ± 5%, 12 h dark/light cycle). All rats had free access to tap water and food.

Experimental design

In our previous study on rats [17, 18], the treatment with 300 000 IU/kg/day and 450 000 IU/kg/day of CMS led to tubular damage. This study aimed to examine the spontaneous renal recovery after stopping CMS and the effect of treatment with vit E.

Animals were randomly divided into 9 groups (n = 6) as follows:

G_1**:** were given 1 ml/kg/day of sterile saline for 7 days and were sacrificed after 12h;

G_2 and G_3**:** received 300 000 IU/kg/day and 450 000 IU/kg/day of CMS for 7 days, respectively, and were sacrificed after 12h;

G_4 and G_5**:** received 300 000 IU/kg/day and 450 000 IU/kg/day of CMS for 7 days, respectively, and were sacrificed after 2 weeks;

G_6 and G_7**:** received 300 000 IU/kg/day and 450 000 IU/kg/day of CMS for 7 days, respectively, were treated with vit E for 2 weeks and sacrificed after 12h;

G_8 and G_9**:** received 300 000 IU/kg/day and 450 000 IU/kg/day of CMS for 7 days, respectively, were treated with olive oil (OO) for 2 weeks and sacrificed after 12h.

Sterile saline and CMS were injected intramuscularly (i.m.) in twice daily doses (12h apart). Vit E was dissolved in 1 ml/kg of OO and injected subcutaneously in once daily dose. The dose of vit E (100 mg/kg/day) had been reported to be effective against nephrotoxicity induced by vancomycin [13].

Preparation of urine, blood and renal tissues samples

At the end of each experiment period, animals were housed in individual metabolic cages and 12h urine samples were collected and centrifuged at 1000 g for 5 min [17, 19]. The supernatant was aliquoted into Eppendorf tubes for determination of NAG level.

Thereafter, animals were anesthetized, euthanized, and blood samples were collected from the heart in heparin tubes and centrifuged at 2500 g for 15 min [18, 20]. The plasma was aliquoted into Eppendorf tubes for determination of Cr level.

Then, the kidneys were removed, 500 mg were homogenized in 5 ml of lysis buffer (50 Mm Tris, 150 mM NaCl adjusted to pH 7.4) and centrifuged at 8000 g for 10 min [18, 21]. The supernatant was collected for the determination of MDA, SOD and GSH levels.

Biochemical assays

Estimation of Cr level

The concentration of Cr in plasma was measured by Jaffe method using commercial diagnostic kits (Ref. 304331) purchased from Biomagreb (Ariana, Tunisia).

Estimation of urine NAG

The concentration of N-acetyl-β-D-glucosaminidase (NAG) in urine was determined by colorimetric assay (Roche Applied Science, 68298 Mannheim, Germany).

Protein quantification

Kidney protein contents were assayed by the method of Bradford [22].

Lipid peroxidation marker in kidneys

The MDA level in renal tissues was determined spectrophotometrically according to Draper and Hadley [23].

Antioxidant markers in the renal tissues

The SOD activity was estimated according to Beauchamp and Fridovich [24] and GSH activity was assayed by the method of Ellman [25] modified by Jollow et al. [26].

Histopathological examination

For light microscopic examination, kidneys removed from the control and tested rats were cleaned and fixed in 10% buffered formalin solution. Then they were embedded in paraffin and stained with hematoxylin–eosin for histopathological studies. All sections were evaluated for the degree of tubular and glomerular injury and necrosis.

Statistical analysis

Data are expressed as mean ± SD (standard deviation). The statistical significance between experimental groups was assessed by one-way analysis of variance (ANOVA) followed by Tukey post-hoc test. Statistical significance was set at $p < 0.05$.

RESULTS

Plasma Cr level

pCr showed no significant change between different experimental groups (Table 1).

Urine NAG level

Urinary NAG levels increased significantly by 29% and 43%, respectively, in groups receiving 300 000 IU/kg/day and 450 000 IU/kg/day of CMS, compared to control. In spontaneous renal recovery the NAG decreased but without significant change. However, the treatment with vit E reduced significantly the level of NAG (Table 1).

Table 1: Variation of plasma Cr and urinary NAG levels in different experimental groups of rats

Group	Plasma Cr (mg/dl)	Urine NAG (U/L)
Control	0.47±0.10	24.31 ± 3.54
7 days after administration of 300 000 IU/kg/day of CMS	0.46 ± 0.08	31.38 ± 4.45*
2 weeks after stopping CMS (spontaneous regeneration)	0.5 ± 0.12	29.56 ± 4.22
2 weeks after treatment with vit E	0.49 ± 0.10	26.08 ± 4.57#
2 weeks after treatment with OO	0.47 ± 0.08	30.21 ± 4.32
7 days after administration of 450 000 IU/kg/day of CMS	0.46 ± 0.07	34.78 ± 4.51**
2 weeks after stopping CMS (spontaneous regeneration)	0.5 ± 0.03	31.22 ± 4.33
2 weeks after treatment with vit E	0.46 ± 0.07	29.22 ± 3.15#
2 weeks after treatment with OO	0.45 ± 0.06	31.82 ± 4.12

Values are expressed as mean ± SD of six rats. *$p < 0.05$ and **$p < 0.01$ vs. control; #$p < 0.05$ vs. CMS group. CMS = colistin methanesulfonate; Cr: creatinine; NAG: N-acetyl-β-D-glucosaminidase, OO: olive oil; vit E: vitamin E.

Lipid peroxidation in kidney

MDA renal tissues levels increased significantly by 34% and 56%, respectively, after administration of 300 000 and 450 000 IU/kg/day of CMS, compared to control. Two weeks

after stopping the CMS, the MDA levels showed a slight decrease. However, the treatment with vit E attenuated significantly lipid peroxidation by 22% and 21%, respectively, compared to the CMS groups (Fig. 1).

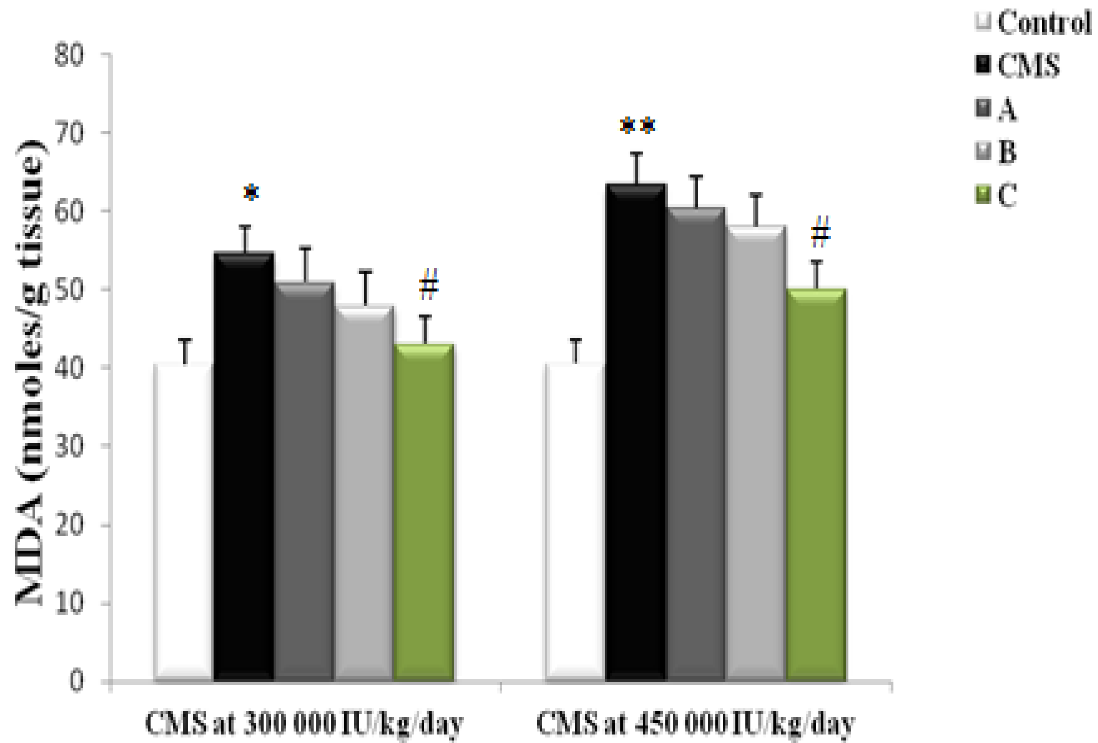

Figure 1: MDA level in kidney of the control, rats exposed to CMS for 7 days, 2 weeks after stopping CMS (**A**), 2 weeks after treatment with OO (**B**) or vit E (**C**). Values are expressed as mean ± SD of six rats. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. control group; $^{\#}p < 0.05$ vs. CMS group.

Antioxidant parameters in kidney

The activities of SOD and GSH in renal tissues declined after administration of CMS, the highest in the 450 000 IU/kg/day group, compared to those of the control. Two weeks after stopping the CMS, the MDA levels showed a slight decrease. However, the treatment with vit E restored these activities, compared to those of the CMS groups (Fig. 2 a-b).

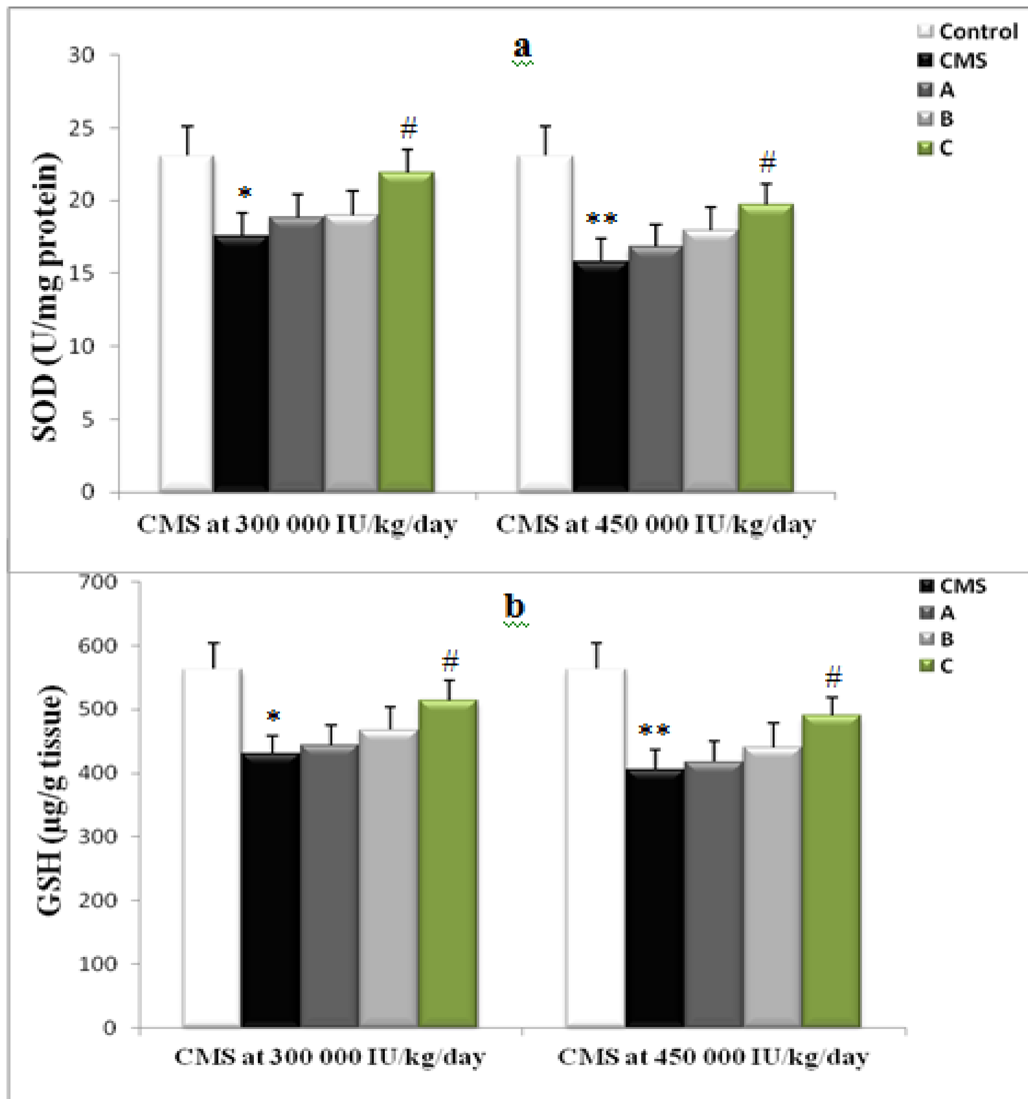

Figure 2: SOD (2a) and GSH (2b) levels in kidney of the control, rats exposed to CMS for 7 days, 2 weeks after stopping CMS (**A**), 2 weeks after treatment with OO (**B**) or vit E (**C**). Values are expressed as mean ± SD of six rats. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. control group; $^{\#}p < 0.05$ vs. CMS group.

Histopathological examination

Light microscopic examinations of the kidneys in the control group revealed a normal structure (Fig. 3A). However, the kidneys of rats receiving 300 000 IU/kg/day of CMS showed slight tubular dilatation (Fig. 3B) and those of the group receiving 450 000 IU/kg/day of CMS demonstrated a severe tubular necrosis (Fig. 3C). After 2 weeks of stopping CMS, renal sections in two groups showed no significant spontaneous renal regeneration (Fig. 3D-E). However, treatment with vit E improved tubular regeneration (Fig. 3F-G).

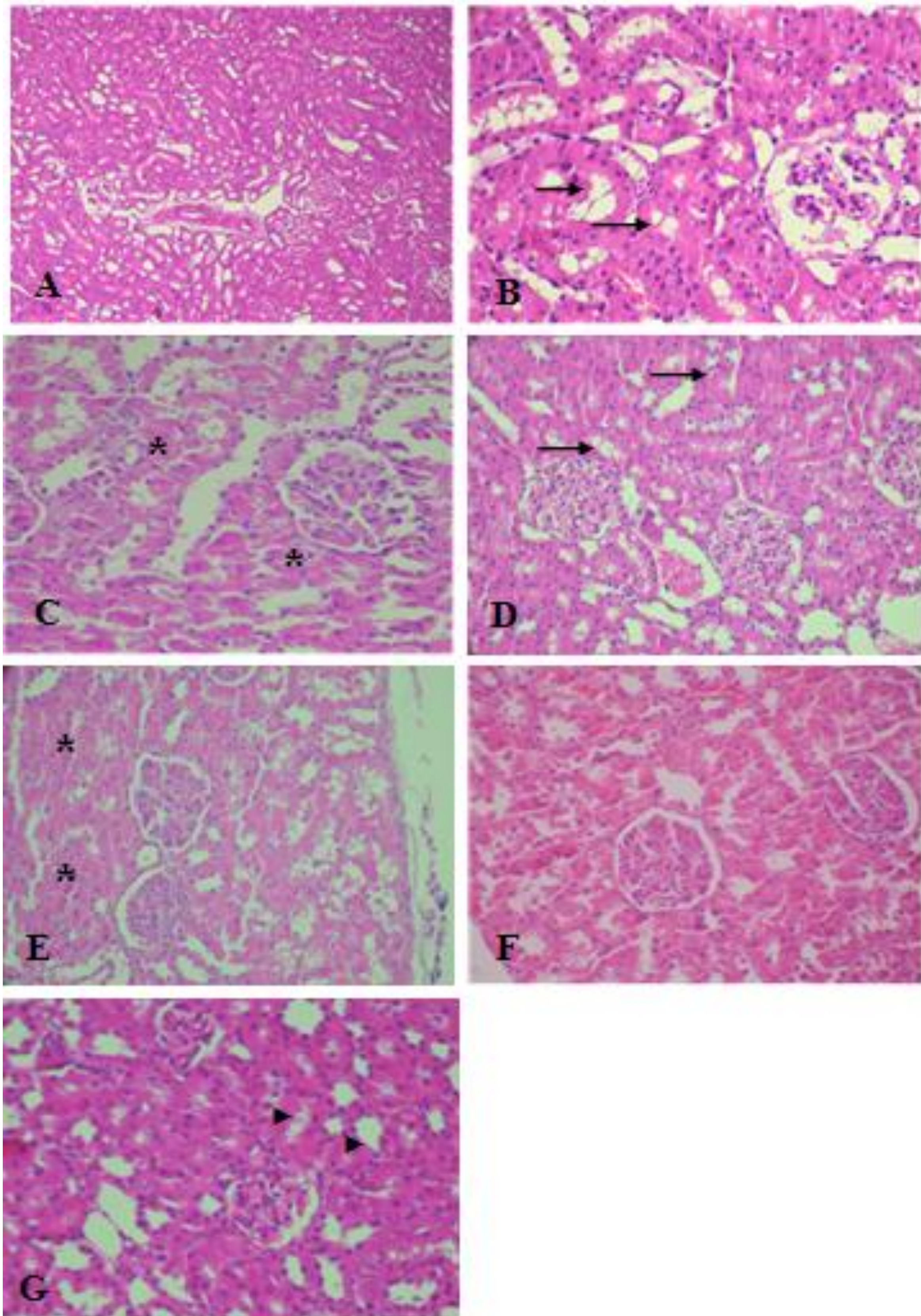

Figure 3: Images of kidney tissue sections of rats: (**A**) receiving saline for 7 days, showed normal renal cortex; (**B**) receiving 300 000 IU/kg/day of CMS for 7 days, showed slight tubular dilatation (➞); (**C**) receiving 450 000 IU/kg/day of CMS for 7 days, showed an acute

tubular necrosis (*); (**D**) 2 weeks after stopping administration of 300 000 IU/kg/day of CMS, showed slight renal recovery; (**E**) 2 weeks after stopping administration of 450 000 IU/kg/day of CMS, showed no significant renal recovery; (**F**) 2 weeks of treatment with vit E following discontinuation of 300 000 IU/kg/day of CMS, showed normal renal histology; (**G**) 2 weeks of treatment with vit E following discontinuation of 450 000 IU/kg/day of CMS, showed a significant reduction of tubular necrosis. Original magnification was H.E (X 100) for panel (A) and H.E (X 250) for all other panels.

DISCUSSION

The administration of 300 000 IU/kg/day of CMS for 7 days led to a slight focal tubular dilatation. The severity of renal damage seems to be more prominent in kidneys of the 450 000 IU/kg/day group, with an acute tubular necrosis. Further, we observed a significant increase of urine NAG, nonetheless plasma Cr remained normal. Urine NAG appears therefore more sensitive than Cr for early detection of proximal tubular damage due to colistin, as mentioned previously [9, 27]. Furthermore, we observed a significant rise in MDA level and a decline of SOD and GSH activities in renal tissue of groups exposed to colistin. Thus, these finding support the implication of oxidative stress in nephrotoxic effect due to colistin; oxidative stress has been reported in nephrotoxicity induced by numerous drugs [28-30]. Indeed, the proximal tubular cells lesions, attested by the rise of urinary NAG, would be the consequence of excessive production of free radical and the exhaustion of antioxidant enzymes.

After two weeks of stopping colistin, spontaneous evolution of tubular damage showed no significant histological improvement. However, urine NAG and oxidative stress markers revealed a tendency to decrease. An eventual restitution of renal tissue would be therefore possible within more than two weeks. Koch-Weser et al. [8] reported that renal dysfunction could progress two weeks after stopping colistin and usually resolved in 3 to 9 weeks. In clinical setting, based on the normalization of pCr, authors estimated that renal reversibility following cessation of colistin, might be resolved in one month or in 5 to 6 weeks [7, 8]. However, pCr is not sensitive enough for better estimation of renal dysfunction; Ghlissi et al. [17]; Yousef et al. [10] and Wallace et al. [6] demonstrated that the administration of high doses of colistin in rats led to severe tubular damage but without change in pCr. Interestingly, the reversibility of interstitial renal damage described by Kallel et al. [31] concerns the immuno-allergic mechanism damage and not the direct toxicity as elaborated here.

If we support the involvement of oxidative stress in nephrotoxic effect of colistin, it would be conceivable to find a tubular damage improvement after antioxidant treatment. Indeed, the treatment for two weeks with vit E, following discontinuation of colistin, revealed a significant renal regeneration. Histological and biochemical recovery seem to be total in the 300 000 IU/kg/day group. Renal amelioration might be therefore due to antioxidant effect of vit E in neutralizing free radicals generated by colistin. Vit E is a fat-soluble antioxidant that can scavenge free radicals and inhibit the propagation of membrane lipid peroxidation [32-33]. The decline of MDA level and the rise of SOD and GSH activities in renal tissue explain the curative effect of vit E. Additionally; the decreased level of NAG indicates the regeneration of tubular cell damage. Thus, the treatment with vitamin E accelerated at least in part the reversibility of renal lesions compared to spontaneous evolution.

We concluded that after two weeks of stopping colistin, the spontaneous renal reversibility showed no significant amelioration. The treatment with vit E improved renal recovery. The curative effect of vit E might be related to reduction of free radicals.

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