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Original Article

The effect of vitamin E in prevention of vancomycin-induced nephrotoxicity in rats

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Abstract

The aim of this study was to investigate the protective role of vitamin E against vancomycin-induced nephrotoxicity. There are some evidences that oxidative injury could be involved in its pathogenesis. Vitamin E at doses of 50, 100 and 200 mg/kg were administered s.c. to rats 30 min prior to i.p. injection of 200 mg/kg vancomycin (VAN). Drug administrations were done every 12 h for 7 days. Afterwards, urine and blood samples were collected and several parameters including activity of urinary γ-glutamyl-transferase (GGT), alanine aminopeptidase and lactate dehydrogenase (LDH), serum urea and creatinine (Cr) concentrations and changes in body and kidney weights were measured. In the animals which received only VAN, the activity of urinary GGT decreased and the activity of LDH in urine increased significantly compared to controls. Serum urea and Cr concentrations and the weight of animals' kidneys increased and body weights decreased significantly in this group compared to controls. Vitamin E at the dose of 200 mg/kg, normalized the GGT and LDH activity. In addition, this dose ameliorated the rise in serum urea and Cr concentrations and improved the changes in kidney and body weights significantly. Other two doses of vitamin E only could modify the changes in body weights. There were marked pathologic changes in tubules of kidneys of VAN treated animals. The tissue injury was prevented only by 200 mg/kg vitamin E, however it remained different from controls. Vitamin E itself did not show any adverse effects on kidneys. It seems that VAN-induced nephrotoxicity might be at least partly due to free radical formation and vitamin E can attenuate its toxicity.

Keywords: Nephrotoxicity; Oxidative injury; Vancomycin; Vitamin E.

INTRODUCTION

Vancomycin (VAN) is a glycopeptide antibiotic which is active against gram positive bacteria including methicillin resistant *staphylococci*. The nephrotoxicity of VAN could limit its dose and duration of administration (1). The mechanism of VAN nephrotoxicity is not fully understood, however some authors have suggested that the mechanism is similar to that of gentamicin (2). Several investigators have suggested that oxygen free radicals are considered to be important mediators of gentamicin-induced nephrotoxicity (3). Investigations on VAN also indicate that free radicals are involved in the pathogenesis of VAN-induced nephrotoxicity, which could be through inhibition of superoxide dismutase (SOD) enzyme (1). In another study, King and Smith demonstrated that VAN can increase

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ATP concentrations of renal cells and stimulate oxygen consumption, supporting the role of VAN as a stimulant of oxidative phosphorylation (4). An increase in oxygen consumption in turn, leads to more free radical generation (5). In addition, some antioxidants like erdosteine (6), α-lipoic acid, *Ginkgo biloba* and melatonin (7) have shown protective effects against VAN-induced nephrotoxicity. It seems that oxidative stress is one of the underlying causes of VAN-induced nephrotoxicity and antioxidant usage may prevent it. In this study we investigated the effect of vitamin E on VAN-induced nephrotoxicity in rats. Vitamin E is a putative radical scavenger which is probably the most important inhibitor of membrane lipid peroxidation. It is a lipid soluble agent which can readily cross cell membranes and exert its effects both intracellularly and in membranes (8). Vitamine E has shown promising effects in gentamicin- (9) cisplatin- (10) and adriamycin-induced nephrotoxicity (11)

MATERIALS AND METHODS

Animals and treatments

Male albino Wistar rats weighing 200-300 g were provided from a local breeding center for this research. The animals were fed with rat chaw and tab water *ad libitum* and kept at 21 ± 3 ºC with a 14 h light/10 h dark cycle. All procedures were checked and approved by the Ethical Committee of the Isfahan University of Medical Sciences, and conducted in accordance with the internationally accepted principles for laboratory animal use and care. The rats were weighed prior to the first injection, every other day in order to adjust the dose according to weight changes and in the 7th day to measure weight changes during the study. VAN was dissolved in normal saline (N/S) in 1:10 solution (w/v) and prepared freshly everyday. Vitamin E solution was prepared in sesame oil. The rats were randomly divided into six experimental groups. In four groups, animal injections of 200 mg/kg VAN i.p. were done every 12 h for 7 days. This dose of VAN was based on the preliminary studies which conducted to find out the appropriate nephrotoxic dose of VAN and was in accordance with two other studies (1,6).

Thirty min prior to each VAN administration, vitamin E was injected s.c. at doses of 50, 100 and 200 mg/kg to either three groups. The injection volume was constant in all groups (0.2 ml/100 g body weight) indicating an almost fixed amount of sesame oil was administered in all groups. The remaining two groups received i.p. administration of N/S. One of these groups was also given 200 mg/kg vitamin E s.c. 30 min before N/S administration. At least 6 animals in each group were used.

On the 7th day after the 14th injection, the animals were placed in plastic metabolic cages at 9 p.m. and the urine free from feces was collected for 12 h. During this time, the animals had free access to water, but because of a possibility of contamination the urine with food particles, the food was removed. After collecting the urine specimens, the animals were anesthetized by ether and then sacrificed through exsanguination and the blood collected into test tubes and both kidneys were removed and weighed. The left kidneys were prepared for pathologic examinations.

Drugs

The following drugs and laboratory kits were used: vancomycin (Alpharma, Denmark); vitamin E (Sigma, Germany); alanine-4-nitroanilide (Merck, Germany); creatinine (Cr) and urea kits (Pars Azmun, Iran); lactate dehydrogenase (LDH) kit (Kimia Pajouhan, Iran) and γ-glutamyl-transferase (GGT) kit (Chem Enzyme, Iran).

Biochemical analysis

Urine and blood samples were centrifuged at 3000 rpm for 20 min. The supernatant was aspirated and kept refrigerated in sealed 1.5 ml tubes. All biochemical measurements were performed within 48 h after sample collections.

Enzyme activity in urine samples was measured directly on the supernatant (2) as follows: alanine aminopeptidase (AAP) activity using alanine-4-nitroanilide as substrate (12); GGT activity according to Szasz method using γ-glutamyl-p-nitroanilide as substrate (13); LDH activity by using a commercial kit in which LDH catalyses the reaction l-lactate + NAD to pyruvate + NADH and the concentration of produced NADH is measured at 340 nm. Urinary enzyme activity was expressed as international units (IU) per lit. To rule out the influence of urinary dilution or concentration (6) or urine flow rate (14), enzyme activity was divided by urinary Cr concentration, so the urinary enzyme excretion was expressed as IU/mg urinary Cr.

The concentration of Cr in both urine and serum samples was measured by Jaffe method and urea in serum by Berthelot method, using commercial kits.

Histopathologic examination

The left kidney of animals was removed from the body and halved through a coronal section. Then the two halves were fixed by immersion in 10% formaldehyde for several days. After processing, they were embedded in paraffin and cut into 3-4 µm slices. The slices were mounted on glass slides and stained with hematoxylin and eosin for light microscopy analysis. The assessment was conducted by a pathologist in a blinded way.

The pathologic changes of the kidneys were recorded using a grading scale of 0 to 4 which was related to a subjective impression of the extent of cortical changes as follows:

0 = indistinguishable from controls

1 = minimal, ≤25% cortex affected

2 = mild, >25% and ≤50% cortex affected

3 = moderate, >50% and ≤75% cortex affected

4 = severe, >75% cortex affected

This grading scale was adapted from Goering et al. (15) with a little modification.

Statistical analysis

All results except for pathologic findings indicated as mean ± SEM. The outliers were excluded by Smirnov-Gravus' rejection test. Statistical significance was determined using one way analysis of variations (ANOVA) followed by Tukey post-hoc. The level of significance retained was $P<0.05$. Pathologic data were assessed by Kruskal-Wallis method followed by Mann-Whitney rank sum test.

RESULTS

Urinary enzyme activity

As depicted in Table 1, vitamin E at the dose of 200 mg/kg could rise the decreased AAP activity induced by VAN ($P<0.01$). Vitamin E alone didn't affect the AAP activity. At two lower doses of vitamin E, AAP activity was significantly lower than in the control group ($P<0.05$, Table 1).

VAN administration at the dose of 200 mg/kg alone, induced a reduction of 60% in urine GGT activity compared to the control group ($P<0.001$, Table 1).

Vitamin E at the dose of 200 mg/kg effectively prevented the decrease in GGT activity ($P<0.001$). The other two doses of vitamin E were not effective and actually showed a profile like VAN alone ($P<0.001$ compared to the controls). Administration of 200 mg/kg vitamin E alone did not produce any changes in GGT activity.

Urine LDH activity in animals which received 200 mg/kg VAN was more than eight folds higher than the control group ($P<0.001$, Table 1). Vitamin E at the dose

Table 1. Effects of different doses of vitamin E on vancomycin-induced changes in kidney functions.

Treatment groups	AAP activity (IU/mg creatinine)	GGT activity (IU/mg creatinine)	LDH activity (IU/mg creatinine)	Kidney weight (g/100g body weight)	Percent change in body weight
Normal Saline	50.4 ± 4.7 (6)	882.3 ± 28.7 (6)	19.3 ± 1.8 (8)	0.31 ± 0.02 (8)	1.3 ± 0.5 (6)
Vitamin E 200 mg/kg	49.8 ± 5.3 (6)	765.1 ± 39.1 (6)	19.5 ± 1.9 (6)	0.28 ± 0.02 (6)	2.5 ± 0.5 (6)
VAN 200 mg/kg + Vitamin E 0 mg/kg	31.6 ± 5.3 (8)	359.4 ± 79.0$^{###}$ (8)	158.3 ± 19.0$^{###}$ (6)	0.79 ± 0.07$^{###}$ (11)	-9.6 ± 1.4$^{###}$ (8)
VAN 200 mg/kg + Vitamin E 50 mg/kg	26.1 ± 3.3$^{#}$ (6)	307.9 ± 38.8$^{###}$ (6)	168.3 ± 14.3$^{###}$ (6)	0.64 ± 0.05$^{##}$ (6)	-2.4 ± 0.5$^{†††,\#}$ (6)
VAN 200 mg/kg + Vitamin E 100 mg/kg	26.9 ± 3.5$^{#}$ (6)	289.1 ± 42.9$^{###}$ (6)	187.0 ± 34.8$^{###}$ (6)	0.68 ± 0.07$^{###}$ (6)	-2.0 ± 0.9$^{†††,\#}$ (6)
VAN 200 mg/kg + Vitamin E 200 mg/kg	58.7 ± 6.5** (6)	1079.0 ± 149.0*** (6)	33.0 ± 3.4*** (6)	0.37 ± 0.02†††,† (6)	3.0 ± 0.9†††,†† (6)

VAN: vancomycin; $^{**}P<0.01$ and $^{***}P<0.001$ versus 3,4,5; $^{\#}P<0.05$, $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ versus 1; $^{†}P<0.05$ and $^{††}P<0.01$ versus 4,5; $^{†††}P<0.001$ versus 3. Numbers in brackets indicate the number of animals used in each group.

of 200 mg/kg prevented the increase in LDH activity ($P<0.001$) but the two lower doses were ineffective in improving the LDH increase ($P<0.001$, Table 1). Vitamin E alone did not affect the LDH activity.

Serum urea and cr concentrations

Both serum urea and Cr concentrations in 200 mg/kg VAN treated animals were respectively 5 ($P<0.001$, Fig. 1) and 2.5 ($P<0.05$, Fig. 2) folds higher than the controls. Vitamin E at dose of 200 mg/kg prevented the increase in serum urea and Cr concentrations ($P<0.001$ and $P<0.05$ respectively). The other two doses could not ameliorate the rise in serum urea and Cr concentrations and even the urea concentration was significantly higher in these two groups than the controls ($P<0.05$, Fig. 1). Application of vitamin E alone did not raise serum urea or Cr concentrations.

Kidney and body weights

The ratio of right kidney weight to 100 g body weight in animals that received 200 mg/kg VAN alone was significantly more than that of the controls ($P<0.001$, Table 1). Vitamin E only at the highest dose, reduced this ratio by 53% ($P<0.001$). The kidney weights in 50 and 100 was significantly higher than controls ($P<0.01$ and $P<0.001$, respectively). The results with vitamin E alone were similar to the controls.

VAN at dose of 200 mg/kg decreased the animal weights during treatment course significantly ($P<0.001$, Table 1). All doses of vitamin E were effective in modifying weight loss in comparison to VAN group ($P<0.001$). Interestingly, in the highest dose, the animals gain weight, like the animals in the control and vitamin E alone treated animals. However, in other two doses, the animals lost weight which was remarkable compared to controls (Table 1).

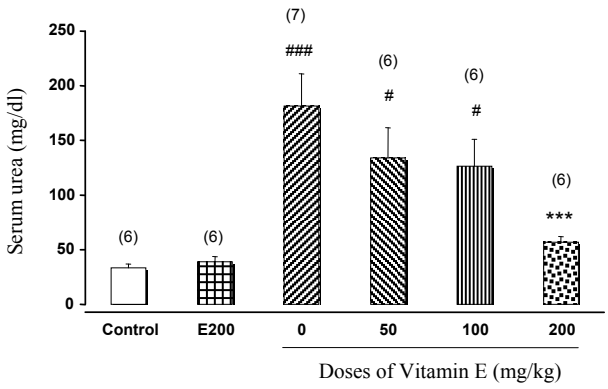

Fig. 1. Effects of different doses of vitamin E on serum urea in VAN-induced nephrotoxicity. All test groups received the indicated dose of vitamin E plus 200 mg/kg VAN. The control group received only normal saline. E200 represents a group of animals which received 200 mg/kg vitamin E alone. Data are expressed as mean ± SEM. $^{***}P<0.001$ versus 0 mg/kg. $^{\#}P<0.05$ and $^{\#\#\#}P<0.001$ versus control. Number of animals in each group is indicated in parentheses above the bars.

Pathological changes

There was no sign of kidney disease in the control and the animals which received 200 mg/kg vitamin E alone. There were no glomerular damages in any group, except an increase in urinary space in some of Bowman's capsules, mainly in animals which received 200 mg/kg VAN.

There were marked pathologic changes in tubules of kidneys of VAN treated animals. Cellular edema, cell necrosis and tubular atrophy together with various types of casts (specially hyaline and polymorphonuclear casts) were the most evident changes. Only the dose of 200 mg/kg of vitamin E prevented from tubular injury, as was evident in relevant tissue slides (P<0.01, Table 2). However, there were tissue abnormalities in all doses of vitamin E (P<0.001 compared to controls and vitamin E alone treated animals). In addition to tubular changes, perivascular infiltration and lymphocytic inflammatory cell infiltration (which was more prominent in lower doses of vitamin E), were seen in tissue slides. In contrast to tissues related to 50 and 100 mg/kg vitamin E, interstitial edema, brush border loss and vacuolization were sparsely seen in slides of animals that were under co-administration of 200 mg/kg vitamin E and VAN. Table 2 summarizes pathologic findings according to grading scale previously described.

Table 2. Pathologic findings in different doses of vitamin E in VAN-induced nephrotoxicity.

	Pathologic grading					
Treatment groups	0	1	2	3	4	n
Normal Saline	8					8
Vitamin E 200 mg/kg	6					6
VAN 200 mg/kg + Vitamin E 0 mg/kg$^{###}$		1	1	4	5	11
VAN 200 mg/kg + Vitamin E 50 mg/kg$^{###}$			2	3	1	6
VAN 200 mg/kg + Vitamin E 100 mg/kg$^{###}$		1	2	2	1	6
VAN 200 mg/kg + Vitamin E 200 mg/kg$^{**,###,\dagger}$		3	3			6

The numbers in the table are representative of the numbers of kidneys existed in each grade in different groups. $^{**}P$<0.01 versus group 3. $^{###}P$<0.001 versus groups 1 and 2. $^{\dagger}P$<0.05 versus group 4. Description of grading scale is as follows: 0 = normal or indistinguishable from controls; 1 = minimal, ≤25% cortex affected; 2 = mild, >25% and ≤50% cortex affected; 3 = moderate, >50% and ≤ 75% cortex affected; 4 = severe, >75% cortex affected. n is number of animals in each group.

DISCUSSION

One of the major adverse effects of VAN is nephrotoxicity which has limited its dose and duration of administration (1). The mechanism of VAN nephrotoxicity is not fully understood, however several studies have proposed that the mechanism of VAN nephrotoxicity might be oxidative injury and could be prevented by use of some antioxidants (1,4,6,7). Vitamin E is a putative antioxidant which exerts its effects through scavenging radicals in lipid phases such as membranes (5). Its efficacy

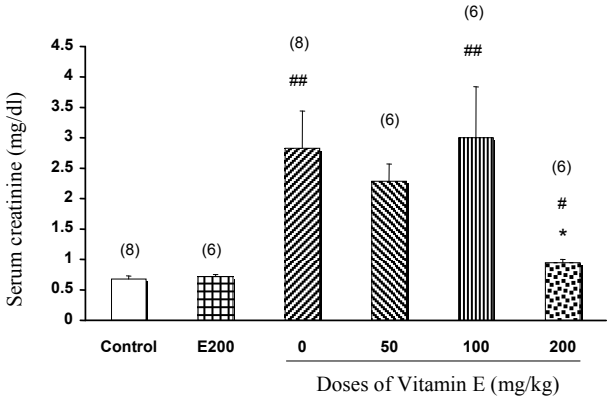

Fig. 2. Effect of different doses of vitamin E on serum creatinine in VAN-induced nephrotoxicity. All test groups received the indicated dose of vitamin E plus 200 mg/kg VAN. The control group received only normal saline. E200 represents a group of animals which received 200 mg/kg vitamin E alone. Data are expressed as mean ± SEM. $^{##}P$<0.01 versus control. $^{*}P$<0.05 versus 0 mg/kg. $^{\#}P$<0.05 versus 100 mg/kg. Number of animals in each group is indicated in parentheses above the bars.

in protecting against kidney oxidative injury induced by gentamicin (9,16), cisplatin (10,17) and adriamycin (11) has been established.

In this study, we investigated the effect of vitamin E on VAN-induced nephrotoxicity in rats. As was mentioned before, vitamin E at the dose of 200 mg/kg, could reverse the increase in urine LDH activity induced by 200 mg/kg VAN. The enzyme elevation is in concert with many other studies in which tubular injury results in urinary LDH elevation. Itoh and colleagues reported that in a 2 week administration of 250 mg/kg/d VAN in rats, there was a significant increase in urinary LDH (18). Hofmeister et al. have suggested that LDH excretion in urine is sufficient for diagnosis of tubular injury in rats (19).

AAP and GGT activity decreased in animals received 200 mg/kg VAN. The difference between this group and controls was significant only in the case of GGT. This decrease in activity is consistent with the findings of Inselmann et al. that following 4 days administration of amphotericin B in rats, AAP and GGT activities in urine was decreased versus time during the study (20). Verweij et al. have also reported that a single dose of mitomycin C caused acute leakage of AAP in rat urine, however chronic administration of the drug resulted in a significant decrease in AAP excretion (21). On the other hand, administration of a single dose of 300 mg/kg VAN to rats, had significantly increased GGT activity in urine after 24 h (2). It seems that a single dose of VAN causes acute leakage of GGT in rat urine, however chronic administration of the drug resulted in a significant decrease in GGT excretion. Vitamin E at the dose of 200 mg/kg could restore the AAP and GGT activities.

Elevations of serum urea and Cr in 200 mg/kg VAN group indicated a reduction in glomerular filtration rate (GFR) and renal failure (22). There were no glomerular damages but extensive tubular lesions were present as indicated by pathological findings. These findings together with elevations in urea and Cr concentrations produced by VAN, leads to a diagnosis of "nephrotoxic acute renal failure" which is classically indicative of acute tubular necrosis (23). In this situation, GFR declines because the flow of glomerular filtrate within tubules is obstructed by casts and necrotic debris and also a backleak of filtrate through injured epithelium exists (23). Vitamin E at the highest dose applied, effectively normalized urea and Cr elevations.

The changes in kidney weights and body weights in 200 mg/kg VAN group were indicative of severe nephrotoxicity, as was reported before (1,24). Vitamin E only at the highest dose, reduced both of these indices. However, the other two doses of vitamin E, were only effective on modifying the decrease in body weights. Since all other parameters indicated that the lower doses studied here were not protective against VAN nephrotoxicity, it seems that the "changes in body weights" is not a reliable index for assessment of nephrotoxicity.

Pathologic findings confirmed the laboratory measurements which were indicative of acute tubular injury induced by VAN. Light microscopic observations revealed that only 200 mg/kg vitamin E could prevent the deleterious effects of VAN on renal tubules, although even this effective dose, as well as other two doses, showed marked tissue abnormalities compared to the controls.

In conclusion, this study revealed that the mechanism of VAN-induced acute tubular injury is at least partly by free radical formation. Vitamin E at the dose of 200 mg/kg resembled to have a protective effect against VAN nephrotoxicity. Vitamin E is the main endogenous antioxidant which reacts with oxygen radicals, preventing free radical chain reactions and protects the membranes

against lipid peroxidation (5,16) and protects from cell damages.

It is proposed to test the protective effect of the other antioxidants in VAN-induced nephrotoxicity.

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