Protective Effects of Aminophylline on Vancomycin-Induced Acute Kidney Injury in Rats: Anti-Oxidant and Anti-Inflammatory Roles

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Abstract

***Background*: Vancomycin is currently the antibiotic of choice for serious infections such as methicillin-resistant *Staphylococcus aureus (MRSA)* and *Enterococcus faecium* but its usefulness is limited by the development of nephrotoxicity. *Aim:* The present study was designed to determine the protective roles of aminophylline in vancomycin-induced acute kidney injury (AKI) in rats. *Materials and Methods:* Male Wister rats (n=40) were used. Vancomycin nephrotoxicity was induced through intraperitoneal injection of vancomycin (200 mg/kg twice daily) for 7 days. Aminophylline was administered once daily in a dose of 24mg/kg alone or combined with vancomycin for 7 days. *Results:* Vancomycin was observed to cause a severe nephrotoxicity, which was evidenced through increased kidney index, elevated blood urea nitrogen (BUN) and reduced creatinine clearance. Renal malondialdehyde (MDA) levels were significantly increased with marked decrease in renal reduced glutathione (GSH) levels as well as renal superoxide dismutase (SOD) and catalase activities. Moreover, elevated renal tumor necrosis factor-alpha (TNF-α) and low renal interleukin-10 (IL-10) levels were observed. Prior aminophylline administration to rats treated with vancomycin showed significant reduction in kidney index and BUN with elevation in creatinine clearance. In addition, aminophylline reduced elevated renal MDA and TNF-α levels and promoted renal GSH levels in addition to renal SOD and catalase activities with upregulation of renal IL-10. *Conclusions:* Oxidative stress and inflammation are remarkable pathways in the pathogenesis of vancomycin induced AKI. Aminophylline could be used in a prophylactic manner in vancomycin associated acute renal injury.**

Key words: Inflammation, Nephrotoxicity, Oxidative stress, Theophylline, Vancomycin

Introduction

Vancomycin, a glycopeptide antibiotic[1], is one of the most frequently used antimicrobials for the treatment of various serious Gram-positive infections, such as *MRSA* and penicillin-resistant pneumococci infections along with other resistances against beta lactam antibiotics[2-4]. The increase incidence of vancomycin-induced AKI is associated with high doses administration[5,6], which are used to reach serum concentrations recommended by the clinical practice guidelines and consensus statement of Infectious Diseases Society of America (IDSA) in the event of treatment failure or resistance[7-9]. Vancomycin nephrotoxicity is associated with reduction in the cellular antioxidant activity leading to oxidative stress dysfunction with generation of reac-

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tive oxygen species (ROS)[6,10]. Reactive oxygen species may produce cellular injury and inflammatory events[5,6,11] with involvement of many cytokines and chemokines including TNF-α, IL-1β, IL-6, IL-8 and interferon-gamma (IFN-γ)[9,12]. IL-10 is a potent anti-inflammatory cytokine that inhibits many inflammatory and cytotoxic pathways involved in AKI; it is capable of inhibiting synthesis of proinflammatory cytokines such as TNF-α, IFN-γ, IL-2, IL-3[9] and protects against ischemic AKI and cisplatin-induced AKI[13]. In the extracellular space, adenosine, a vital component of the energy-producing machinery, acts as a signaling molecule during conditions of inflammation or acute injury[14,15]. In the kidney, four adenosine receptors (ARs) can be identified, the A1ARs, A2AARs, A2BARs and A3ARs[14,16]. Inflammation, ischemia and hypoxia altered adenosine receptor expression significantly[14,16]. Despite the significant medical challenge of AKI, therapeutic approaches to prevent or treat vancomycin-induced AKI are limited with a significant interest in developing novel therapeutic approaches[16]. Theophylline and aminophylline, a mixture of theophylline and ethylenediamine, are non-selective adenosine receptors antagonists and are non-selective phosphodiesterase (PDE) isoenzymes inhibitors[17]. They are widely used in the treatment of bronchial asthma and chronic obstructive pulmonary diseases[18]. Theophylline increases IL-10 release through PDE enzymes inhibition[18,19]. Aminophylline has been reported to possess free radical scavenging effects[18,20]. In rats, aminophylline ameliorated cisplatin-induced renal failure through inhibition of A1ARs[21,22]. In addition, theophylline and other methylxanthines ameliorated the experimental acute renal failure caused by glycerol[23-25]. However, the preventive potentials of aminophylline in the regulation of ROS/inflammatory/immune-related functions in vancomycin induced acute nephrotoxicity have been less well studied. So, this study was designed to elucidate the possible preventive roles of aminophylline in vancomycin induced rat acute kidney injury.

Materials and Methods

All experimental protocols were approved by the Institutional Animal Care and Use Committee at the Suez Canal University following the National Institutes of Health guide for the care and use of laboratory animals (Maryland, USA).

Experimental animals

Forty adult male albino Wistar rats weighing 220-250gm were obtained from the Egyptian Organization for Biological Products and Vaccines (Egypt). Rats were kept under controlled laboratory conditions of normal light-dark cycle and temperature 25±3°C and allowed for acclimatization for one week before the start of the study.

Drugs and chemicals

Vancomycin (Vancocin®, 500mg vial, Eli Lilly, Cairo, Egypt), dissolved in distilled water, was injected intraperitoneally (I.P.) at a dose of 200 mg/kg twice daily for 7 days, which is the dosage scheme reported to cause marked nephrotoxicity in rats[6,10]. Aminophylline (Minophylline-N®, 125mg/5ml/ampoule, Alexandria Co. for pharmaceuticals, Alexandria, Egypt) was administered at a dose of 24 mg/kg I.P once daily for 7 days[13]. Vancomycin was administered 30 minutes after the first dose of aminophylline[22]. All other chemicals were purchased from Sigma-Aldrich Company, St. Louis. U.S.A. Manipulation of the animals was done under light ether anaesthesia.

Experimental design

The rats were weighed and randomly divided into 5 groups consisting of 8 rats per

group. Group (1): Control untreated group, in which rats were received no medications. Group (2): Vehicle-control group, where rats were received distilled water I.P. 12th hourly for 7 days. Group (3): Vancomycin-control group, in which rats were received saline I.P. once daily and vancomycin twice daily for 7 days. Group (4): Aminophylline control group, where rats were received once daily aminophylline only for 7 days. Group (5): Aminophylline & vancomycin treated group, in which rats were received daily aminophylline and vancomycin for 7 days. On the 8th day, rats were weighed again and then were placed individually in metabolic cages to collect the 24 hours urine samples where they were continued to have free access to water and food. Before scarification, blood samples were collected from individual rats of all groups through retro-orbital plexus under ether anaesthesia. After scarification by decapitation, kidneys were dissected out and were washed in cold normal saline. Each right kidney was weighed to calculate kidney weight/body weight ratio (kidney index) and then was subjected to the histopathological examinations. Each left kidney was used for lipid peroxidation, GSH, SOD and catalase assays and for the renal TNF-α and IL-10 contents.

Biochemical Analysis

The blood and urine samples were centrifuged. Plasma was aspirated, together with the urine specimens, then BUN with serum and urine creatinine levels were assayed using Hitachi auto-analyser 912. Creatinine clearance was calculated, after estimating serum and urine creatinine as reported previously according the equation: Creatinine clearance (ml/min)= Creatinine in urine (µmol/L) X Urine volume (ml/24h)/ Serum creatinine (µmol/L) X 1440[26].

Preparation of tissue homogenate

Each left kidney was separated into 2 parts. Each part was weighed. One part was homogenized in ice-cold Tris buffer (pH 7.4) to give a 10% w/v homogenate, which was centrifuged at 15.000 rpm at 4°C for 15 minutes. The supernatant was stored at -80°C for SOD, catalase, GSH and lipid peroxidation assays[6,10,27]. The remaining part was homogenized in phosphate buffer saline (PBS). The homogenate suspension was centrifuged at 14,000 rpm for 10 min at 4°C and the supernatant was kept at -80°C until analysis for kidney TNF- α and IL-10[28].

Lipid peroxidation assay

The assay was based on the principle that the end product of lipid peroxidation MDA forms a 1:2 adduct with thiobarbituric acid and the red pigment produced was extracted with nbutanol-pyridine mixture and estimated by the absorbance at 532 nm. MDA was expressed in nmol per milligram of protein[29].

Reduced Glutathione assay

Renal GSH measurement was based on the principle that reduced glutathione interacts with 5-5'dithiobis 2-nitrobenzoic acid (DTNB) to form the colored product 2-nitro-5-thiobenzoic acid, which was measured at 412nm and expressed in nmol per milligram of protein[10].

Superoxide dismutase assay

In renal homogenates, SOD activity was measured as previously described[10,28] with a kit from Randox, Antrim, UK using a UV-visible spectrophotometer (UV-1601PC, Shimadzu, Japan) and expressed in units per milligram of tissue protein.

Catalase assay

Principle: the catalase enzyme could catalyze the decomposition of hydrogen peroxides to water and oxygen; in which the decomposition of peroxide was measured at 240nm. The catalase activity was expressed as units/mg of tissue protein[6,10].

Renal TNF-α and IL-10 measurements

Renal homogenate contents of TNF-α and IL-10 were measured by an automated enzyme-linked immunosorbent assay (ELISA) reader (Metertech, M960) using rat TNF-α and IL-10 ELISA kits (Ray Biotech Inc®, Norcross, USA).

Histopathological examinations

Right Kidneys were fixed in 10% formalin, dehydrated with ethyl alcohol and then embedded in paraffin wax. Sections were stained with haemotoxylin and eosin and examined under a light microscope with high power (40X). According to the extent of cortical changes, a grading scale of 0 to 4 was used: Grade 0= no changes detected; Grade 1=minimal, ≤25% cortex affected; Grade 2=mild, >25% and ≤50% cortex affected; Grade 3=moderate, >50% and ≤75% cortex affected; Grade 4=severe, >75% cortex affected[27].

Statistical analysis

Results were collected and expressed as Mean±standard deviation (SD) then analyzed using the Statistical Package for the Social Sciences, version 20 (SPSS Software, SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) followed by Duncan's post-hoc test were used to test the significance between quantitative variables. $P<0.05$ was considered statistically significant.

Results

Effects on kidney weight/body weight ratio

Kidney weight/body weight ratio was significantly higher with vancomycin administration (0.61±0.13 mg/g) compared to the control untreated group (0.32±0.06 mg/g, $P<0.05$). Aminophylline co-administration reduced significantly this increased ratio (0.44±0.11 mg/g, $P<0.05$) in comparison with vancomycin control group (Figure 1),.

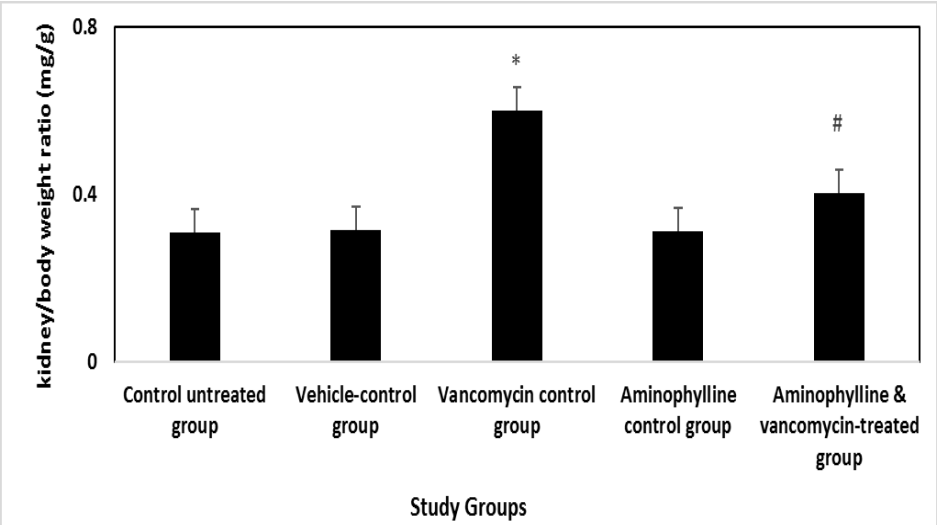

Figure 1: Effects of vancomycin and aminophylline on kidney weight/body weight ratio (mg/g). Values are expressed as the mean±SD and analyzed by one-way ANOVA followed by Duncan's multiple comparisons test, (n=8) *****$P<0.05$ compared with control untreated group, $^{\#}P<0.05$ compared with vancomycin control group

Effects on BUN, serum creatinine and creatinine clearance

In vancomycin control group, the BUN and serum creatinine levels were significantly increased in comparison with the control group. These elevations were significantly reduced by aminophylline administration compared to the vancomycin control group ($P<0.05$). Creatinine clearance was decreased significantly with vancomycin

treatment compared to the control untreated rats. Concomitant administration of aminophylline and vancomycin was significantly restored these values nearly to values obtained in normal rats, in comparison to monotherapy with vancomycin (Table 1).

Table 1: Effects of vancomycin and aminophylline on blood urea nitrogen, serum creatinine, creatinine clearance, renal MDA and GSH levels in addition to renal SOD and catalase activities

Groups	Control untreated group	Vehicle-control group	Vancomycin control group	Aminophylline control group	Aminophylline & vancomycin-treated group
BUN (mg/dl)	38.63 ± 7.85	40.24 ± 6.73	62.48 ± 8.78*	39.42 ± 5.02	46.01 ± 4.23 #
S. Creat. (mg/dl)	0.41 ± 0.16	0.42 ± 0.18	0.93 ± 0.26*	0.41 ± 0.19	0.58 ± 0.24 #
Creat. clearance (ml/min)	3.54 ± 0.21	3.73 ± 0.28	1.09 ± 0.13*	3.42 ± 0.29	3.25 ± 0.17 #
MDA (nmol/mg protein)	1.47 ± 0.58	1.68 ± 0.47	9.63 ± 0.86*	1.54±0.32	2.22± 0.91 #
GSH (nmo/mg protein)	0.43 ± 0.11	0.42 ± 0.15	0.21 ± 0.08*	0.59 ± 0.21*	0.38 ± 0.11 #$
SOD (U/mg protein)	25.42 ± 4.85	23.95 ± 6.12	10.73 ± 2.65*	41.34 ± 5.76*	24.31±5.02 #$
Catalase (U/mg tissue)	1.42 ± 0.36	1.39 ± 0.24	0.87 ± 0.24*	1.94 ± 0.33*	1.31 ± 0.27 #$

BUN, blood urea nitrogen, S. Creat.: serum creatinine, MDA: malondialdehyde, SOD: superoxide dismutase, GSH: reduced glutathione. Values are the mean±SD, n=8. *$P < 0.05$ compared with control untreated group, #$P < 0.05$ compared with vancomycin control group, $$P < 0.05$ compared with aminophylline control group.

Effects on kidney MDA concentration, SOD, GSH and catalase activities

A statistically significant elevation in the lipid peroxidation end product MDA levels were considered with vancomycin administration in comparison with the control untreated group (Table 1). Co-administration of aminophylline and vancomycin was resulted in significant reductions in mean renal MDA concentrations compared to vancomycin monotherapy ($P<0.05$; Table 1). Vancomycin injections were associated also with significant reduction in renal GSH levels as well as renal SOD and catalase activities compared to the control untreated group ($P<0.05$; Table 1). These reductions were almost returned to the concentrations measured in the control untreated group by aminophylline treatment in comparison to vancomycin control group (Table 1). Of notice, aminophylline administration was accompanied by significant elevations in the antioxidant markers GSH level and SOD and catalase activities compared to the control untreated group (Table 1).

Effects on kidney TNF-α and IL-10 concentrations

Statistically significant elevations in the mean kidney TNF-α concentration (76.30± 2.42 pg/ml) was assessed after vancomycin administration in comparison with the control untreated group (29.62±1.84 pg/ml, $P<0.05$). These elevations were significantly reverted by treatment with aminophylline (36.84±3.16pg/ml) compared to vancomycin control group (Figure 2A). The concentrations of IL-10 significantly decreased in rats treated with vanco-mycin (9.84±3.27 pg/ml) compared to the control untreated group (25.13±4.01 pg/ml). Significantly, aminophylline monotherapy was associated with elevation in the kidney concentrations of this cytokine (48.26±3.96 pg/ml) compared with the control untreated rats. In rats co-treated with aminophylline and vancomycin, the concentration of IL-10 (39.23±3.17 pg/ml) was significantly elevated compared to the rats treated with vancomycin alone and to the control untreated rats (Figure 2B).

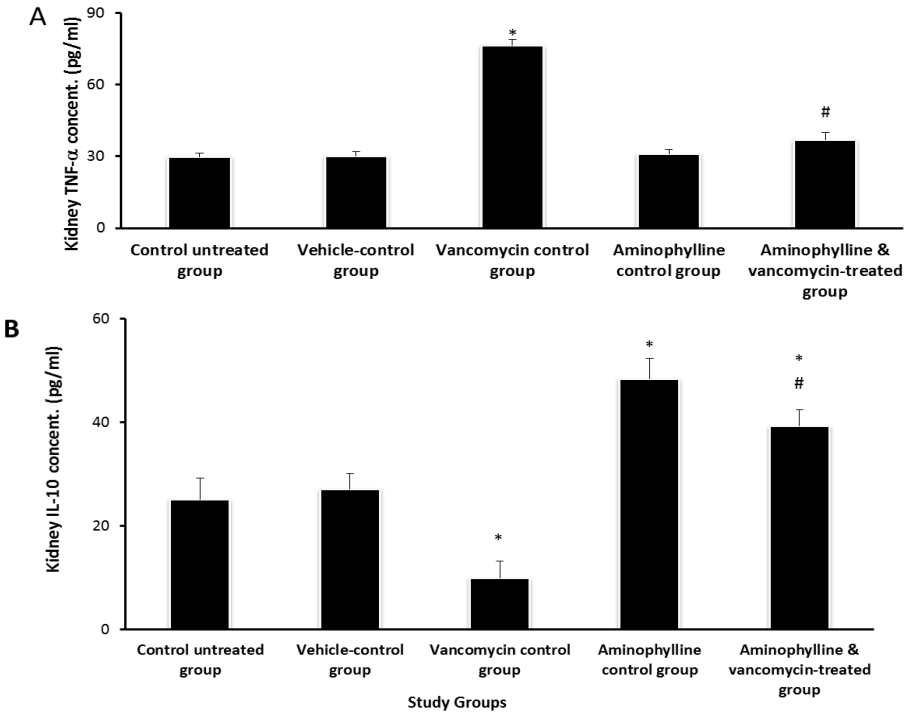

Figure 2: Effects of vancomycin and aminophylline on (A) kidney TNF-α concentrations (pg/ml) and (B) kidney IL-10 concentrations (pg/ml). Values are expressed as the mean±SD and analyzed by one-way ANOVA followed by Duncan's multiple comparisons test (n=8). **P* <0.05 compared with control untreated group, #*P* <0.05 compared with vancomycin control group.

Effects on histopathological changes and scoring

Regarding the histopathological sections (Figure 3), the control untreated group, the vehicle-control group and the aminophylline control groups showed no obvious renal histological alterations (Figure 3I; A, B, D and Figure 3II). Conversely, significant deterioration was observed in the vancomycin control group manifested as epithelial desquamation, epithelial vacuolization, tubular distortion, tubular necrosis, tubular dilatation, interstitial edema and inflammatory cell infiltration (Figure 3I; C) with a mean histopathological score of 3.17±0.19 (*P*<0.05; Figure 3II). Aminophylline administration significantly (*P*<0.05) improved the histopathological changes associated with vancomycin by reversing most of the tubular changes with glomeruli of minimal inflammation, tubular distortion, epithelial vacuolization and desquamation displaying a mean histopathological score of 1.31±0.22 (Figure 3I; D and 3II).

Discussion

In different animal studies with vancomycin therapy, the role of free radicals and oxidative stress on the renal tubules[30,31] with alterations in many cytokines such as TNF-α, IL-10[9,12,13], signaling molecules as adenosine[14,15], and inflammatory cascades has been recognized[32-34]. Determining the mechanism of vancomycin-associated nephrotoxicity is important to potentially develop methods to prevent this adverse event[35].

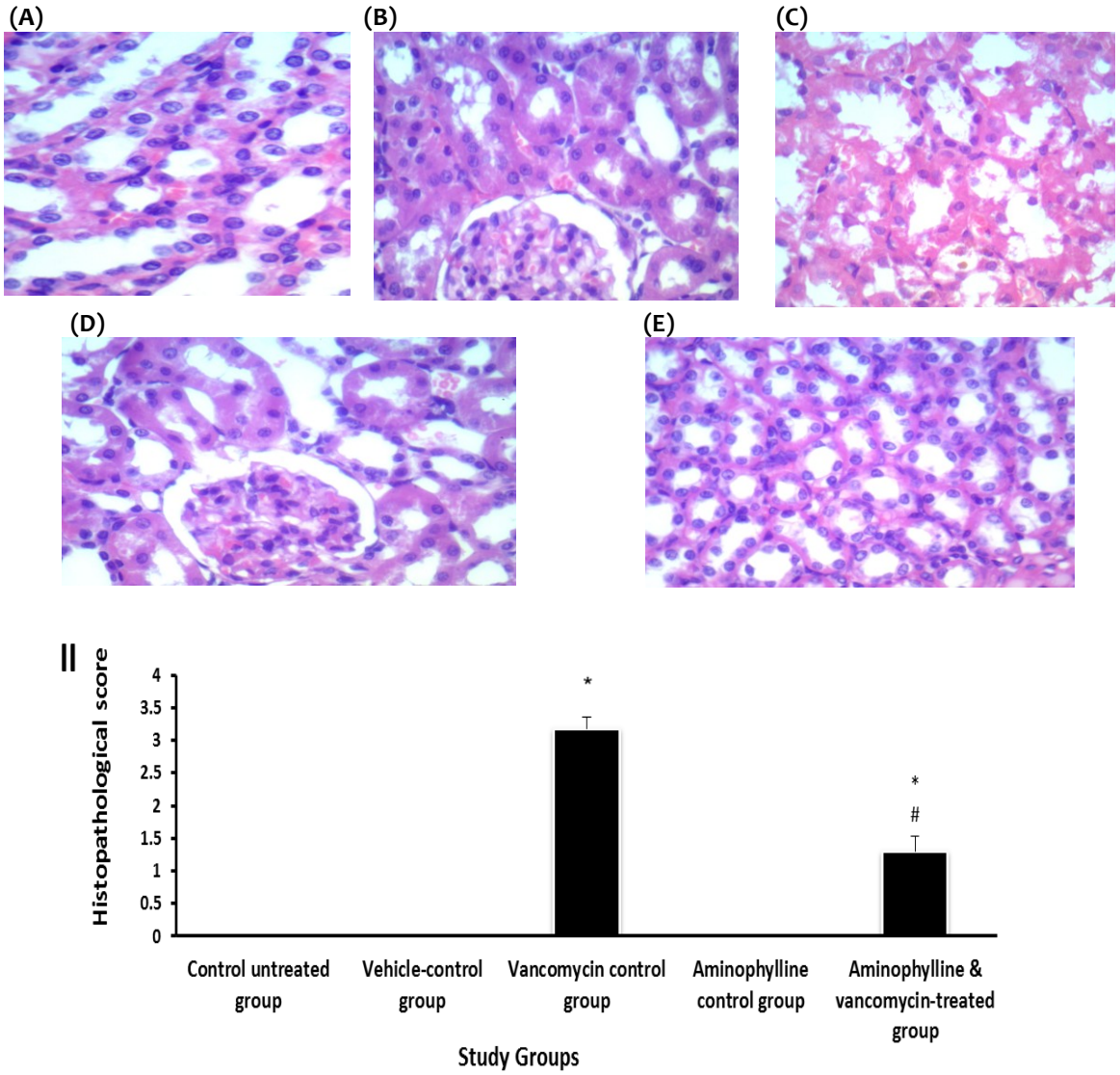

Figure 3: (I): Histopathological changes of kidneys in experimental groups; (H&E 40X). Control-untreated group (A), vehicle control group (B), vancomycin control group (C), aminophylline control group (D) and aminophylline plus vancomycin treated group (E). Photomicrographs A, B and D showed normal renal tissue architecture. Epithelial desquamation with vacuolization, tubular distortion, necrosis and dilatation and interstitial edema with inflammatory cell infiltration were observed with vancomycin (C). Aminophylline reversed vancomycin-associated renal tissue deleterious findings (E). (II): Histopathological scores in the experimental groups. Data was expressed as means±S.D and analyzed by one-way ANOVA followed by Duncan's multiple comparisons test (n=8). $^{*}P<0.05$ compared with control untreated group, $^{\#}P<0.05$ compared with vancomycin control group.

In the current study, aminophylline was assessed experimentally to prevent vancomycin-induced acute kidney injury. Results of the present study revealed that vancomycin administration markedly increased the kidney weights/body weights ratio, serum creatinine and BUN levels with noticeable reduction in creatinine clearance. In consistence with the earlier studies, these findings were indicated a reduction in the glomerular filtration rate leading to a diagnosis of "nephrotoxic acute renal failure" which is classically indicative of acute tubular necrosis[10,27,32,34]. Aminophylline dissoci-

ated into theophylline in biological fluids and since theophylline is an adenosine receptor antagonist and non-selective PDE isoenzymes inhibitor, it was attributed to antagonize the hemodynamic effects of endogenous adenosine in the impairment of glomerular filtration rate[17,21,36]. Our results demonstrated that aminophylline significantly decreased the rise in kidney weight/body weight ratio, BUN and serum creatinine. In addition, it prevented the fall in creatinine clearance associated with vancomycin use. Reactive oxygen species have been proposed as the causative factors of the renal damage of vancomycin[5,6,33,36]. The body possesses defense mechanisms such as antioxidants to limit free radical damage. These protective agents are consisting of enzymatic species including SOD, GSH and catalase enzymes[5,6,10,36]. MDA is formed during oxidative degeneration and is accepted as an indicator of lipid peroxidation[5,6,33]. The present study demonstrated that vancomycin-treated rats showed high renal levels of MDA and low renal SOD, GSH and catalase activities. Aminophylline has been reported to possess free radical scavenging, anti-inflammatory and lipid peroxidation reduction effects[18,20,37]. In consistency, our outcomes revealed that aminophylline has been shown to reduce renal MDA levels and elevate renal SOD, GSH, and catalase activities, when administered before vancomycin. Remarkably, our results showed that aminophylline monotherapy increased the SOD, GSH, and catalase activities in normal renal tissues. IL-10 is known to suppress inflammatory responses[26] and to guard against ischemic AKI and cisplatin-induced AKI[13]. Theophylline increases IL-10 release through PDE enzymes inhibition[18,19], which approved by our findings that administration of aminophylline to normal rats induced a significant rise in renal IL-10 concentrations. Regarding our data, vancomycin treated rats showed a significant fall in renal IL-10 levels. Moreover, co-administration of aminophylline and vancomycin improved significantly the vancomycin-induced reduction in IL-10 levels. IL-10 is capable of inhibiting synthesis of proinflammatory cytokines such as TNF-α, IL-2, IL-3 and IFN-γ[9]. In accordance, our study showed that renal TNF-α levels was significantly elevated with vancomycin administration and concomitant administration of aminophylline reversed the elevation in this pro-inflammatory cytokine. These outcomes were allied well with the histopathological findings reported in the present study, which revealed severe distortion in the renal cortex mainly with tubular necrosis in rats treated with vancomycin. Similar changes were also reported by Celik et al., 2005, Ocak et al., 2007, Panonnummal et al., 2011 and Basarslan et al., 2012 who demonstrated severe structural injurious changes in renal tissue after vancomycin administration[5,6,10,32]. In several animal models with toxic AKI, aminophylline ameliorated the existence of nephrotoxicity revealed by improvement in the unfavorable histological changes[22,23]. In consistence with theses earlier studies, prior aminophylline administration reduced deleterious glomerular and tubular epithelial changes shown in nephrotoxic changes associated with vancomycin.

Conclusion

This work provided direct evidences that oxidative stress and inflammation are remarkable pathways in the pathogenesis of vancomycin induced AKI, highlighting the effects of IL-10 and TNF-α. Aminophylline provided renoprotective effects via antioxidant actions and improvement of the anti-inflammatory markers. Therefore, the present results postulated that aminophylline could be considered in the prevention of

vancomycin-induced AKI. However, any reconsideration about the use of aminophylline would require detailed review of the clinical safety data with a range of doses.

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Conflict of interest: None

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