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Vitamin E protection against gentamicin-induced nephrotoxicity in rats: a biochemical and histopathologic study

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Summary

The specificity of gentamicin for vitamin E deficiency-associated oxidative stresses in the renal proximal convoluted tubules is apparently related to its ability to increasingly facilitate generation of radical species in mitochondria. To determine the ways in which vitamin E manage the currently processes, we conducted a prospective study aimed to investigate the tubular preserving effect of vitamin E, pre-treatment and co-treatment, in nephrotoxicity with gentamicin-treated Sprague-Dawley rats. 35 healthy rats were ascribed 1–5 trials to receive once daily intramuscular injections of either gentamicin (80 mg/kg/body Wt) (GN), normal saline (NS), vitamin E (250 mg/kg/body Wt) (VE), vitamin E (250 mg/kg/body Wt) plus gentamicin (80 mg/kg/body Wt) simultaneously (CGE), or vitamin E alone (250 mg/kg/body Wt) 3 days before co-administration with gentamicin (80 mg/kg/body Wt) (PGE), for 10 days. Gentamicin alone caused a decrease in glomerular filtration rate-associated coefficient of the creatinine clearance, increase in blood content of BUN as well as a decrease in tubular function evident by recognised depression of ATPase activity, increases in lipid peroxidation and subsequently MDA activity. The histopathologic studies revealed acute tubular necrosis with tubular cast formation triggered by gentamicin treatment over 10 days of experiment and change in size and pattern of tubules. Further biochemical studies showed tubular preserving effect of vitamin E pre-administration including slow down in rising enzyme activity (MDA) and mild to moderate BUN with recovery in creatinine clearance and holding ATPase activity up to 50% on comparison with the control and vitamin E alone-treated rats. Significant tubular resistance against gentamicin proximal tubular lesions on the suppressed activity of lipid oxidation induced by vitamin E pre-treatment with normal size during microscopic inspections lead us to conclude protective role of vitamin E is probably attributed to tubular prevention, whereas hyperemia prepared by vitamin is only a consequent.

Key words: Gentamicin, Nephrotoxicity, Vitamin E, Rat

Introduction

Routine therapeutic use of aminoglycoside, gentamicin (80 mg/kg/body Wt) for more than seven days, has long been the commonest cause of nephrotoxicity in approximately 30% of patients (Moore *et al*., 1984; Barclay and Begg, 1994; Pedraza-Chaverrı *et al*., 2003). The specificity of gentamicin for renal toxicity is apparently related to its ability to increasingly facilitate the generation of radical species, including superoxide anions, hydrogen peroxides and hydroxyl radicals in mitochondria, a few of which appears to be a crucial part of the antioxidant deficiency-associated oxidative stresses in the renal proximal convoluted tubules (Maldonado *et al*., 2003; Yanagida *et al*., 2004). Recently, a number of studies demonstrating reduced plasma concentration of endogenous chain-breaking antioxidant, like vitamin E, in nephrotoxicity and interactions between this vitamin and biochemical reactions such as cortical lipid peroxidation, synthesis of radical-driven metabolites and electron-transferring

pathway, suggest that disturbed metabolism of vitamin E may be important in the pathogenesis of nephrotoxicity with gentamicin (Ademuyiwa *et al*., 1990; Abdel-Naim *et al*., 1999; Kadkhodaee *et al*., 2004). Although little information is now available on concentration of vitamin E in tissues which develops the nephrotoxic complications, there is a clear relationship between decrease in gentamicin-induced lipid peroxidation (MDA) and the gross cellular alterations upon dietary administration of vitamin E (250 mg/kg/body Wt) (Elfarra *et al*., 1994; Halliwell and Gutteridge, 1999; Mingeot-Leclercq and Tulkens, 1999; Pedraza-Chaverrı *et al*., 2003). To determine the ways in which pre-treatment and co-treatment of vitamin E manage the currently processes, this study was carried out.

Materials and Methods

Drugs

Vials of both, injectable (i.m.) gentamicin sulphate and vitamin E, each containing 80 mg/2 ml, and 500 mg/1 ml, respectively assigned for medical applications were purchased from Darugostar Co. (Tehran, Iran).

Experiment protocol

Thirty-five young male Sprague-Dawley rats, 8–9-week-old, weighing 200–250 g, were randomly assigned to 1–5 trials of seven rats each received once daily intramuscular injection of either gentamicin (80 mg/kg/body Wt) (GN), normal saline (NS), vitamin E (250 mg/kg/body Wt) (VE), vitamin E (250 mg/kg/body Wt) plus gentamicin (80 mg/kg/body Wt) simultaneously (CGE) or vitamin E alone (250 mg/kg/body Wt) three days before co-administration with gentamicin (80 mg/kg/body Wt) (PGE), for 10 days. One week prior to any treatments, animals were housed and acclimatised to temperature (22 ± 2°C) and humidity (70–75%) in the controlled room with a 12:12 hr light:dark cycle and free access to standard rodent chow (Pars Karadj®, Karadj, Iran) and water. This protocol was performed according to the guide for the care and use of laboratory animals of the Animal Welfare Act (Regulations 9CFR, Parts 1, 2 and 3, as described in the Guide for the Care and Use of Laboratory Animals).

Biochemical assay

In the day off upon the treatment period, all rats were put in individual metabolic cages for collection of 24 hrs urine. Blood (10 am) was drawn out, by punching the vein plexus of the retro-orbital sinus under ether euthanasia, into the polyethylene tubes containing heparin as anticoagulant and stored at -20°C until assay. After 10 min of low-speed (2500 rpm, 4500 × g) centrifugation, the plasma was subjected for measurement of BUN concentration (GLDH-glutamate dehydrogenase enzyme-kinetic method, Stanbio Urea Nitrogen, Liqui-UV®) by means of light spectrophotometer (Beckman DU-50, Fullerton®, Canada). Thereafter, serum and urine creatinine concentrations were measured by alkaline picrate method (Bartels *et al*., 1972), endogenous creatinine clearance (ml/min) over last 24 hrs was calculated through the standard formula. The serum concentrations of sodium and potassium were determined by flame-photometer (M129, Systronic®, Germany). A spectrum analyzer (747, Hitachi®, Japan) with reagent kits (Zist Chemi®, Tehran, Iran) was on instruction set up to measure out plasma concentration of phosphorus and calcium in blood. Determination of lipid peroxidation activity in the derived plasma was based on method of thiobarbituric acid (Bernheim *et al*., 1948). Since this method measures the malondialdehyde (MDA), the reactive products obtained in the final result were expressed as MDA equivalent.

Histopathologic examination

At the end of experiment (on day 11), sodium pentobarbital (200 mg/kg) was administered intraperitoneally to euthanize each rat. The abdominal cavity was immediately opened. The kidneys were dissected out, washed and fixed in 10% neutrally buffered formalin for three days. They were then paraffin embed following the routine procedures. Five-micron sections were prepared and stained with haematoxylin and eosin for examination

under light microscope (Houghton *et al*., 1978).

Statistical analysis

Results are expressed as mean and standard error of the mean (SEM). The significance of differences between the groups was performed using one-way analysis of variance (ANOVA) followed by multiple comparison test. P-value less than 0.05 was considered significant.

Results

Biochemical analysis

Ten days of treatment with gentamicin (80 mg/kg/body Wt) produced remarkable nephrotoxicity, characterized by an increase in blood urea nitrogen (BUN) when compared with the control rats. Vitamin E, whether in pre-treated or concurrent rats, with gentamicin failed to significantly hold the BUN up with normal baseline, however, prior administration of vitamin E showed that it mildly to moderately reversed the changes to control of BUN. There was no significant difference in plasma BUN between the rats received either vitamin E only or normal saline (Table 1).

Daily consecutive administration of gentamicin in the GN rats led to statistically significant decrease in the creatinine clearance as compared with the control group. In double-treated rats, in those which received vitamin E three days before administration of gentamicin, the glomerular filtration was significantly recovered to near baseline compared to rats which received simultaneous vitamin E and gentamicin. There was no significant alteration from control of creatinine perfusion between blank rats and those rats which were only given single daily regimens of vitamin E (Table 1).

Following gentamicin infusion, a comparable increase in fractional sodium excretion occurred in rats received gentamicin alone than the control group (76.77 ± 3.2 vs. 136.86 ± 6.5, respectively). The baseline fractional sodium excretion of pre-treated rats was near two-fold than that of concurrent treated ones. Moreover, bivariate analysis revealed that rats in the VE group did not exhibit alteration in serum sodium concentration in the absence of gentamicin as well as control (Table 1).

After 10 days of therapy, serum potassium reabsorption was significantly ($P<0.05$) reduced in gentamicin alone-treated rats compared with control rats. The mean serum potassium level was significantly ($P<0.05$) increased in pre-treated rats receiving vitamin E prior to gentamicin as compared with rats receiving gentamicin and vitamin E simultaneously. Serum potassium of rats in the VE group was the same as that in the control (Table 1).

Gentamicin alone-treated rats manifested statistically significant rise in the urinary phosphorus and calcium output. Supplemented vitamin E with gentamicin, both in the CGV and in PGV groups, failed to reverse the excretion of phosphorus and calcium almost entirely. Vitamin E alone-treated rats did show no difference from the control group in regards to plasma phosphorus and calcium content (Table 1).

Lipid peroxidation in the renal cortex, as assessed by the accumulation of MDA, was augmented by gentamicin administration but it was significantly decreased in CGV and PGV groups. Pre-administration of vitamin E significantly ($P<0.05$) depressed the total level of MDA, more than concurrent

Table 1: Changes in BUN, creatinine clearance, sodium, potassium, calcium, phosphorus, MDA concentration and urine volume on day 10 (n=10)

Parameter	Mean ± SEM				
	1st Group	2nd Group	3rd Group	4th Group	5th Group
BUN (mg/dl)	44.17±4.2	173.6±7.7	43.71±3.1	85.67±5.0	60.8±3.4
Creatinine clearance (ml/min/100 gr)	0.47±0.08	0.052±0.007	0.46±0.05	0.24±0.03	0.35±0.1
Na (mEq/L)	136.86±6.5	76.77±3.2	130±6.1	90.12±4.5	121.4±5.8
K (mEq/L)	7.61±1.8	4.04±1.1	7.50±1.3	5.24±1.4	7.01±1.6
Ca (mEq/L)	5.79±1.3	3.42±0.9	5.87±1.1	5.14±0.7	5.42±1.1
P (mEq/L)	8.54±2.1	8.39±2.4	8.72±2.0	8.41±2.2	8.51±2.3
MDA (nmoles/ml plasma)	1.23±0.08	2.75±0.15	1.26±0.09	2.31±0.11	1.42±0.09
Urine volume (ml/24 hr)	8±1.0	19±1.6	10±1.0	16±1.3	12±1.1

administration of the drug. In the VE group, vitamin E lowered per-oxidative reactions of lipids over 10 days of treatment in respect to the control (Table 1).

Histopathologic evaluation

The kidneys of rats in both the NS and VE groups were normal. Tissue evaluation in rats given 10-day consecutive administration of gentamicin revealed interstitial nephritis and extensive hyperemia with progressive acute tubular necrosis (ATN) and cast formation resulted from tubular epithelial loss. The histomorphology of kidneys in the CGV displayed mild to moderate epithelial degeneration of renal tubules with wide hyperemia in cortex, while inspection of histopathologic markers relating to nephrotoxicity in the PGV rats showed normal pattern with localised hyperemia (Figs. 1 and 2).

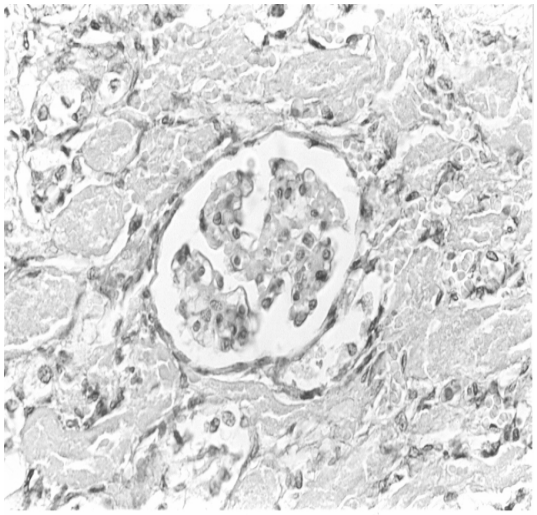

Fig. 1: Acute tubular necrosis in the kidney. Group GN (H&E, ×400)

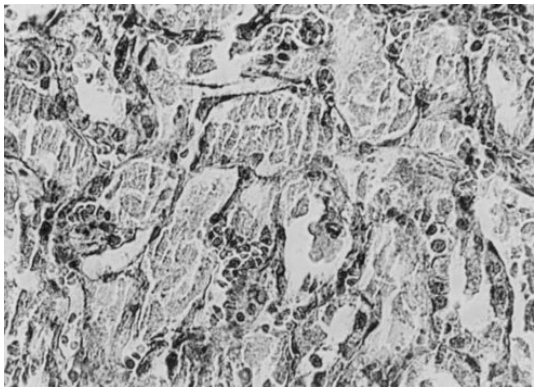

Fig. 2: Mild epithelial degeneration of kidney tubules. Group CGV (H&E, ×400)

Discussion

Results of this study corroborated the previous reports in which gentamicin at dose of 80 mg/kg/body Wt significantly produced nephrotoxicity (Abdel-Gayoum *et al*., 1994; Elfarra *et al*., 1994). Studies showed that primary retention of gentamicin in proximal tubular cells following production of oxygen-associated metabolites and free radicals precede gentamicin-induced nephrotoxicity (Fantone and Ward, 1982; Fox, 1984; Ueda *et al*., 1995). In the present study, we investigated the effects of pre-treatment and co-treatment of vitamin E, a potent antioxidant, on acute renal failure with gentamicin administration in rat.

BUN, serum creatinine concentration and creatinine clearance

After intramuscular administration for up to 10 days, gentamicin (80 mg/kg/body Wt) alone caused a significant reduction in GFR, glomerular changes and secondary tubular casts evident by significant increase in serum BUN and decreased creatinine clearance (Schentag *et al*., 1979; Luft and Evan, 1980; Schor *et al*., 1981; Neugarten *et al*., 1983), whilst pre-treatment of rats with vitamin E gave rise to increased changes in nephrotoxicity on day 10, between the groups receiving concurrent gentamicin + vitamin E and those receiving pre-treatment with vitamin E + gentamicin (Abdel-Naim *et al*., 1999; Sener *et al*., 2003). This was particularly marked by significant changes in BUN concentration. In contrast, these observed differences were paralleled with the possible involved mechanisms, get enhanced primary therapy with vitamin E in preventing of nephrotoxicity to concurrent therapy and were often including contraction of mesangial cells through producing thromboxane A_2 (Parra *et al*., 1998), cytosolic up-regulation of phospholipid A_2 and cyclooxygenase-1, which eventually leads to the release of prostacyclin, a potent vasodilator and inhibitor of platelet aggregation in human (Monsen, 2000).

Sodium, potassium, calcium and phosphorus

Fukuda *et al*. (1991) explicitly showed the electrolyte abnormalities upon treatment

of rats with gentamicin (80 mg/kg/body Wt). Such immediate formation of disturbance further supports the notion in which inactivation of Na/K ATPase is a very early event during interaction of gentamicin with proximal tubular cells. It also indicates that simultaneous inhibition of very different membrane protein species is not necessarily a prerequisite for the initial depression of Na/K ATPase and afterwards, multifactorial cell death processes (Beauchamp *et al*., 1985; Fukuda *et al*., 1991). Relevant effects of vitamin E therapy prior to gentamicin administration in the studies that the activity of Na/K pumps in cell membrane have been regulated suggest a striking association between oxidative pathways, hyper-homocystinemia, suppression of or decrease in glomerular synthesis of thromboxane B_2, O_2-, MDA, H_2O_2, antidiuretic potential of vitamin E (Ademuyiwa *et al*., 1990) and scavenging of free radicals due to turnover of glutathione and vitamin C (Ognjanovic *et al*., 2003). Moreover, some of recent published researches believe that the majority of vitamin E function is probably attributed to tocopherol to prevent the propagation of free radical reactions by acting as a peroxyl radical scavenger and protecting polyunsaturated fatty acids within membrane phospholipids and in plasma lipoproteins (Monsen, 2000).

Remarkable observations of Elliott and Patchin offered in (1992) a well-define theory, in which hypocalcemia has been recognized of subsequent intracellular events between either inhibition of basolateral calcium ATPase, Na/K ATPase or blockage of intraluminal calcium channels and competition of gentamicin with calcium for binding brush border. In addition to conjugated hypo-phosphatemia in gentamicin-treated rats, it was known to eliminate the problems of consequent nephrocalcinosis with the hypocalcemia (Reeves *et al*., 1993).

Lipid peroxidation

A correlation between development of nephrotoxicity and the progression of oxidative stresses has been well-demonstrated in many experimental animal models. Lipid peroxidation and its subsequent products, MDA, are typical examples of oxidation-indicating reactions in nephrotoxicity, both of which were claimed to be causes of irreversible cell damages (Washio *et al*., 1994; Kumar *et al*., 2000). The role of lipid peroxidation in gentamicin-induced acute renal failure has also been described (Ramsammy *et al*., 1987) by evaluating the protective effect of vitamin E so that administration of super oxide dismutase, vitamin E or vitamin C significantly reduced the nephrotoxic symptoms produced by adriamycin (Okasora *et al*., 1992; Washio *et al*., 1994; Kumar *et al*., 2000). Because of its lipophilic nature with minimum toxicity and potent antioxidant property, vitamin E may play an important role as a nephroprotective agent against gentamicin-induced renal impairment (Giuliano *et al*., 1984; Ademuyiwa *et al*., 1990; Abdel-Naim *et al*., 1999). The antioxidant property of vitamin E at level of tubules is probably mediated by enhances of superoxide dismutase and glutathione peroxidise activity or even increase in catalase contents of kidney tissue (Hirano *et al*., 1991; Kavutcu *et al*., 1996; Ozturk *et al*., 1997). Gentamicin enhances the production of hydrogen peroxide in isolated mitochondria (Nakakuki *et al*., 1996). Pre-treatment with vitamin E has been proved to suppress lipid peroxidation pathway as effective as preventing the rise of MDA (Hirano *et al*., 1991). Weglicki *et al*. (1984) demonstrated the inhibition of lipid peroxidation and maintenance of lysosomal latency immediately after treatment of rats with vitamin E. They concluded that preserving effect of vitamin E in stabilizing, exert through off-binding vitamin to membrane phospholipids on its lipophilic properties. Later studies showed that concurrent treatment of rats with vitamin E plus gentamicin for six days did not have any significant effects on the gentamicin-induced alterations of malon-dialdehyde, superoxide dismutase, catalase or the glutathione cascade, however, the shift from polyunsaturated to saturated fatty acids largely reversed. In rats, pre-treated with vitamin E for six days, gentamicin failed to raise renal cortical malondial-dehyde above that of saline-treated rats. The changes in the esterified fatty acids were almost prevented entirely, and there were no significant change from control of the

glutathione cascade. The depressions of superoxide dismutase and of catalase, however, were not reversed (Ramsammy *et al*., 1987). Ramsammy *et al*. (1987) displayed that the pre-treatment of anti-oxidants such as vitamin E drastically facilitated diffusion of vitamin E into lyso-somal area, reduced the lipid peroxidation and MDA inducing shift from poly-unsaturated to the saturated fatty acids in the biological membranes.

Histopathologic findings

Histopathological inspections of GN group supported the biochemical results indicating morphological changes in the renal cortex evident by injuries in cells lining around segments of S1 and S2 of proximal tubules, whereas administration of vitamin E or normal saline alone for 10 days did not cause alteration in renal function. However, co-treatment with vitamin E in rats prevented the renal lesions with gentamicin though moderate tubular changes were observed. Histopathologic results also showed minimal changes in renal tissue, indicating the influence of vitamin E pre-treatment against gentamicin-induced nephrotoxicity. The present biochemical and histologic results supported each other strongly while, some of pervious experimen-tal models have shown that vitamin E cannot prevent or even reduce the severity of gentamicin-induced proximal tubular cell necrosis (Ramsammy *et al*., 1987).

In conclusions, the present study provides evidence that pre-treatment of vitamin E can prevent both the functional and histological renal changes induced by gentamicin in rats.

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