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Protective Effects of Vitamin E and Vitamin C on Gentamicin-Induced Nephrotoxicity in Sheep

[1]Mohammad Mashayekhi and [2]Mohammad Reza Valilou
[1]Department of Clinical Science, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[2]Department of Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran

Abstract: Gentamicin is a commonly used antibiotic medication and this is an aminoglycoside antibiotic, used to treat many types of bacterial infections, particularly those caused by gram-negative organisms. Gentamicin can also be highly nephrotoxic, particularly if multiple doses accumulate over a course of treatment or in dehydrated cases. The aim of this study was to investigate the protective role of vitamin E against Gentamicin-induced nephrotoxicity in sheep. Eighteen healthy Ghezel sheep were randomly divided into three groups of control and treatments with vitamin C and E. All sheep of two groups were received 20 mg/kg/body wt. of Gentamicin every 8 h by intramuscular injection for 10 days. Treatment group were also receive 250 mg/kg/body wt. of vitamin E and C daily by intramuscular injection for 14 days. This study revealed that the GM-induced renal toxicity as measured by multiple functional, structural and enzymatic factors is significantly reduced by co-supplementation of vitamins C and E. The results of this study suggest the potential of antioxidant vitamins to protect against GM-induced nephrotoxicity.

Key words: Vitamin E and C, gentamicin, nephrotoxicity, sheep, antioxidant vitamins, Iran

INTRODUCTION

Gentamicin (GM) is an aminoglycoside antibiotic which is used in clinical practice to treat severe gram-negative infections. However, its nephrotoxic action has limited the extent of its use (Mingeot-Leclercq and Tulkens, 1999). Routine therapeutic use of aminoglycoside, gentamicin (80 mg/kg/body wt.) for >7 days has long been the commonest cause of nephrotoxicity in approximately 30% of patients (Moore *et al*., 1984; Barclay and Begg, 1994; Pedraza-Chaverri *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-Chaverri *et al*., 2003).

There have been many studies in recent years suggesting a significant role for Reactive Oxygen Species (ROS) in GM-induced nephrotoxicity (Cuzzocrea *et al*., 2002). Sha and Schacht (1999a) have suggested that aminoglycoside antibiotics can stimulate formation of free radicals. In addition, ROS scavengers and antioxidants are used to ameliorate the GM-induced nephrotoxicity (Mazzon *et al*., 2001; Maldonado *et al*., 2003). Superoxide dismutase treatment has shown some promise in protection against GM-induced nephrotoxicity in rats (Ali and Bashir, 1996). Recently, it has been shown that both vitamins E and C decreased lipid peroxidation and augmented the activity of antioxidant enzymes in the

Corresponding Author: Mohammad Mashayekhi, Department of Clinical Science, Tabriz Branch, Islamic Azad University, Tabriz, Iran

kidneys of diabetic rats (Kedziora-Kornatowska *et al*., 2003). Prior vitamin E dietary supplementation suppresses oxidative stress and glomerulosclerosis in rat remnant kidney (Hahn *et al*., 1999). Single-dose administration of vitamin E had protective effects on cisplatin-induced nephrotoxicity in developing rats (Appenroth *et al*., 1997). There have been several studies in recent years suggesting more effectiveness of combination therapy by co-supplementation of two antioxidants (Ademuyiwa *et al*., 1990; Kavutcu *et al*., 1996; Abdel-Naim *et al*., 1999). In a state of oxidative damage in rat erythrocytes induced by chlorpyrifos-ethyl, combination of vitamins C and E reduced lipoperoxidative effects (Gultekin *et al*., 2001). The present study aimed to update the current knowledge of the efficacy of these antioxidants against Gentamicin-induced nephrotoxicity in sheep.

MATERIALS AND METHODS

Experimental protocol: This study was conducted in Tabriz during Summer 2011. Vials of both, injectable (i.m.) gentamicin sulphate, vitamins E and C each containing 20 and 250 mg mL^{-1}, respectively assigned for medical applications were purchased from Drugstore Co. (Tabriz, Iran). Eighteen healthy Ghezel sheep divided into three groups including control, treatment with vitamin E and treatment with vitamin C.

Biochemical assay: Urine and blood samples were obtained on days 0, 7, 10, 14 and 17 from sheep of each group and values of creatinine, uric acid, BUN, urea and GGT were measured by special kits.

Statistical analysis: The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), Version 13.0 was used for statistical analysis. All data are presented as mean±SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene-tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Data associated with biochemical parameters on day 0 are as shown in Table 1. Data associated with biochemical parameters on days 7, 10, 14 and 17 in the normal control group are as shown in Table 2. Data associated with biochemical parameters on days 7, 10, 14 and 17 in the treated group with vitamin C are as shown in Table 3. Data associated with biochemical parameters on days 7, 10, 14 and 17 in the treated group with vitamin E are as shown in Table 4.

Aminoglycoside antibiotics including GM can produce nephrotoxicity in human. Proximal tubular cells are a major site of damage in patients treated with GM or the antibiotic amikacin (Wiland and Szechcinski, 2003). GM binds to the cell wall phospholipids, blocking the chain reactions of phosphatidyl inositol which impairs cell integrity (Walker and Duggin, 1988). It has been shown that aminoglycoside antibiotics exert their adverse renal effects by generation of ROS. Formation of ROS following bioactivation of GM has been reported (Sha and Schacht, 1999b).

Some studies demonstrated that antioxidant administration have ameliorated GM-induced nephropathy (Pedraza-Chaverri *et al*., 2003; Atessahin *et al*., 2003). A role for superoxide in GM-mediated nephropathy was suggested when different superoxide dismutase treatments were shown to be effective in ameliorating renal injury in GM-induced nephrotoxicity (Ali and Bashir, 1996; Cuzzocrea *et al*., 2002).

Table 1: Biochemical parameters analysis on day 0 of study

Parameters No.	Creatinine (mg dL^{-1})	Uric acid (mg dL^{-1})	BUN (mg dL^{-1})	Urea (mg dL^{-1})	GGT (IU L^{-1})	Urine casts	Dipstick testing
1	1.11	0.48	18.45	39.4	16	Negative	Normal
2	1.12	0.45	19.11	40.8	18	Negative	Normal
3	1.11	0.46	19.15	40.9	20	Negative	Normal
4	1.09	0.51	18.85	40.3	18	Negative	Normal
5	1.14	0.53	18.90	40.4	16	Negative	Normal
6	1.11	0.46	19.50	41.7	15	Negative	Normal
7	1.08	0.50	19.35	41.4	19	Negative	Normal
8	1.12	0.51	18.45	39.4	18	Negative	Normal
9	1.11	0.45	18.85	40.3	17	Negative	Normal
10	1.07	0.51	19.22	41.1	18	Negative	Normal
11	1.13	0.46	20.31	43.4	19	Negative	Normal
12	1.11	0.47	18.45	39.4	21	Negative	Normal
13	1.12	0.47	18.85	39.8	17	Negative	Normal
14	1.14	0.45	19.20	40.9	18	Negative	Normal
15	1.14	0.49	19.55	41.8	19	Negative	Normal
16	1.07	0.46	19.82	42.4	19	Negative	Normal
17	1.09	0.48	18.25	39.0	18	Negative	Normal
18	1.11	0.51	20.68	44.2	19	Negative	Normal

Table 2: Biochemical parameters analysis of normal control group on days 7, 10, 14 and 17

Parameters No.	Creatinine (mg dL^{-1})	Uric acid (mg dL^{-1})	BUN (mg dL^{-1})	Urea (mg dL^{-1})	GGT (IU L^{-1})	Urine casts	Dipstick testing
Day 7							
1	3.4	1.1	35	74.9	31	Negative	Normal
2	3.3	1.2	32	68.4	35	Epithelial cells	Normal
3	2.8	1.0	28	59.9	27	Negative	Normal
4	2.8	1.2	30	64.2	30	Epithelial cells	Normal
Day 10							
1	3.9	1.8	51	109.1	40	Normal	Normal
2	4.0	1.9	46	98.4	32	Epithelial cells	Normal
3	4.0	1.7	48	102.7	37	Normal	Normal
4	3.7	1.7	49	104.8	43	Epithelial cells	Normal
Day 14							
1	6.3	2.2	54	115.5	46	Epithelial cells	Normal
2	6.4	2.3	53	113.4	45	Epithelial cells	Normal
3	6.0	2.3	51	109.1	42	Normal	Normal
4	6.1	2.0	51	109.1	50	RBC	Hb
Day 17							
1	6.4	2.3	60	128.4	52	Epithelial cells	Normal
2	6.3	2.2	58	124.1	48	Epithelial cells	Normal
3	6.4	2.3	59	126.2	45	Normal	Normal
4	6.2	2.3	59	126.2	53	RBC	Hb

Table 3: Biochemical parameters analysis of treated group with vitamin C on days 7, 10, 14 and 17

Parameters No.	Creatinine (mg dL^{-1})	Uric acid (mg dL^{-1})	BUN (mg dL^{-1})	Urea (mg dL^{-1})	GGT (IU L^{-1})	Urine casts	Dipstick testing
Day 7							
1	2.7	0.9	28	59.9	22	Normal	Normal
2	2.6	0.8	27	57.7	23	Normal	Normal
3	2.7	0.9	27	57.7	21	Normal	Normal
4	2.5	0.7	28	59.9	22	Normal	Normal
Day 10							
1	3.0	1.1	30	64.2	27	Epithelial cells	Normal
2	2.9	1.2	28	59.9	20	Normal	Normal
3	3.0	1.1	29	62.0	33	Normal	Normal
4	3.1	1.1	30	64.2	16	Normal	Normal
Day 14							
1	5.0	1.3	32	68.4	26	Epithelial cells	Normal
2	4.9	1.4	30	64.2	34	Normal	Normal
3	5.1	1.0	28	59.9	18	Epithelial cells	Normal
4	5.0	1.2	30	64.2	24	Normal	Normal
Day 17							
1	4.7	1.1	31	66.3	25	Epithelial cells	Normal
2	4.6	1.2	28	59.9	33	Epithelial cells	Normal
3	4.6	1.2	29	62.0	20	Epithelial cells	Normal
4	4.7	1.2	29	62.0	23	Normal	Normal

Table 4: Biochemical parameters analysis of treated group with vitamin E on days 7, 10, 14 and 17

Parameters No.	Creatinine (mg dL^{-1})	Uric acid (mg dL^{-1})	BUN (mg dL^{-1})	Urea (mg dL^{-1})	GGT (IU L^{-})	Urine casts	Dipstick testing
Day 7							
1	2.2	0.7	24	51.3	21	Normal	Normal
2	2.3	0.6	26	55.6	26	Normal	Normal
3	2.2	0.8	25	53.5	17	Normal	Normal
4	2.2	0.7	24	51.3	20	Normal	Normal
Day 10							
1	2.1	1.0	28	59.9	22	Normal	Normal
2	2.4	1.1	30	64.2	30	Epithelial cells	Normal
3	2.2	0.9	29	62.0	19	Normal	Normal
4	2.1	1.0	30	64.2	18	Normal	Normal
Day 14							
1	4.0	1.2	29	62.0	24	Epithelial cells	Normal
2	4.1	1.3	27	57.7	32	Epithelial cells	Normal
3	3.9	1.1	30	64.2	21	Normal	Normal
4	4.0	1.3	27	57.7	19	Normal	Normal
Day 17							
1	3.9	1.1	29	62.0	23	Epithelial cells	Normal
2	3.8	1.2	26	55.6	32	Epithelial cells	Normal
3	4.0	1.1	27	57.7	19	Normal	Normal
4	3.9	1.1	28	59.9	19	Normal	Normal

In another study, lipid peroxide levels were reduced and levels of antioxidant enzymes and thiol compounds were increased following administration of α-tocopherol and ascorbic acid in lead-induced oxidative stress (Patra *et al*. 2001). 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, researchers investigated the effects of pretreatment and co-treatment of vitamin E, a potent antioxidant, on acute renal failure with gentamicin administration in rat.

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).

This study revealed that the GM-induced renal toxicity as measured by multiple functional, structural and enzymatic factors is significantly reduced by co-supplementation of vitamins C and E. The results of this study suggest the potential of antioxidant vitamins to protect against GM-induced nephrotoxicity.

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