RESEARCH ARTICLE

A Study of Oxidative Stress in Gentamicin Induced Nephrotoxicity and Effect of Antioxidant Vitamin C in Wistar Rats

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Received: 28.06.2012
Accepted: 31.07.2012

DOI: 10.5455/njppp.2013.3.14-20

ABSTRACT

Background: Gentamicin is very useful aminoglycoside in treatment of gram negative organisms but nephrotoxicity has been a major limiting factor. Various renal pathologies have shown correlation with generation of oxidative stress. So, it was hypothesized that there may be involvement of oxidative stress in gentamicin nephrotoxicity.

Aims & Objective: To investigate the role of oxidative stress in gentamicin-induced nephrotoxicity and its prevention by using antioxidant vitamin C in Wistar albino rats. Another objective was to localize the site of kidney damage produced by gentamicin.

Materials and Methods: Male Wistar albino rats were administered gentamicin at the dose of 70 mg/kg/day i.m; either alone or in combination with vitamin C. Parameters of oxidative stress like glutathione peroxidase (GPx), glutathione reductase (GR) and total antioxidant status (TAS) were analysed. Renal damage was measured by blood urea and serum creatinine. Histopathological examination of rat kidney was done. The data was subjected to one way ANOVA with post hoc test of significance and the values of $P < 0.05$ were considered statistically significant.

Results: Treatment with gentamicin caused significant reduction of TAS, GPx and GR activity, while blood urea and serum creatinine level were raised significantly as compared to control. Renal damage was confirmed with histopathological studies. The primary site of damage was identified as tubules especially proximal convoluted tubules. Administration of vitamin C alone raised TAS level significantly. Co-administration of vitamin C along with gentamicin led to significant restoration of GPx, GR, TAS, blood urea and serum creatinine level.

Conclusion: Renal damage due to gentamicin is associated with oxidative stress. The primary site of damage was renal proximal convoluted tubules. Co-administration of vitamin C along with gentamicin significantly prevented nephrotoxicity by virtue of its antioxidant effect.

KEY WORDS: Gentamicin; Nephrotoxicity; Vitamin C; Glutathione Peroxidase; Glutathione Reductase; Total Antioxidant Status

INTRODUCTION

Gentamicin is a well-established aminoglycoside antibiotic for the treatment of infection caused by gram-negative organisms. However, nephro-toxicity has always been a limiting factor in the therapeutic application of gentamicin. It imposes a ceiling on the total dose as well as the total length of the treatment and even necessitates substantial reduction in the dose in patients with compromised renal function. Nephrotoxicity has been traced to be due to marked accumulation and retention of aminoglycosides in the proximal convoluted tubular cells.[1]

Oxidative stress has been recognized as an important contributory factor in a number of pathogenic processes including those affecting kidney[2-4] leading to the possibility of utilizing the antioxidants for the prevention of nephrotoxicity.

Accordingly this study was planned to investigate the possible generation of oxidative stress by gentamicin leading to renal damage in experimental rat model. Also it was aimed at localization of the site of damage produced by gentamicin with histopathological study of rat kidney.

Antioxidants are substances capable to repair or prevent the damage produced by oxidative stress. Vitamin C is a naturally occurring powerful antioxidant. It has shown beneficial effect in many conditions where oxidative stress is generated.[5,6] So the study was also aimed to explore the possible effect of antioxidant vitamin C for combating the renal damage produced by gentamicin.

MATERIALS AND METHODS

Adult male Wistar albino rats weighing 150 g – 200 g were used in the study. The animals were kept in the vivarium and fed animal house diet with water ad libitum. Experiment was conducted after receiving approval from the animal ethical committee. The animals were divided into four equal groups randomly, each group comprising 7 rats. The treatment schedule was as follows:

Group I served as a control and received saline (0.3 ml of i.p.) daily.
Group II received vitamin C (0.2 mg/kg/day i.p.).
Group III was administered gentamicin (70 mg/kg/day i.m.).
Group IV received gentamicin (70 mg/kg/day i.m.) and vitamin C (0.2 mg/kg/day i.p.).

All the treatments were given as a single daily dose for thirty days. The nephrotoxic dose of gentamicin was decided by conducting a pilot study.

Sample Collection

At the end of treatment, the rats were sacrificed after giving sodium thiopentone 50 mg/kg i.p. and blood samples were collected directly from the heart. The blood samples were taken in plain as well as heparinized bulbs for biochemical estimation. The kidneys were dissected out and preserved in 10 % formalin for the histopathological study.

Biochemical Estimations

Markers of renal impairment: Blood urea and serum creatinine were measured from collected samples.

Markers for the free radical involvement: Glutathione peroxidase (GPx), glutathione reductase (GR) and total antioxidant status (TAS) levels were estimated in serum. After clot formation the samples were centrifuged at 2000 rpm for 5 minutes and serum was separated. The kits were purchased from Randox Laboratory Limited, Diamond road, Crumlin, Co. Antrim, U.K. The analysis of GPx, GR and TAS was done on spectrophotometer.

The enzyme GPx was assayed in each sample by using method described by Paglia and Valentine.[7] GR catalyses the reduction of oxidized glutathione (GSSG) in the presence of NADPH, which is oxidized to NADP+. The decrease in the absorbance is measured at 340

nm.[8] TAS was measured as described in the method of Miller et al.[9]

Statistical Analysis

The data was subjected to one way ANOVA with post hoc test of significance and the values of $P < 0.05$ were considered statistically significant. The data was analysed with the help of software Open EPI.

RESULTS

Glutathione Peroxidase Enzyme Activity

In vitamin C group there was rise of about 9% in the GPx activity. In gentamicin group the GPx activity was significantly decreased. In group where vitamin C was co-administered the reduction in GPx activity was significantly less marked (Table-1).

Glutathione Reductase Enzyme Activity

In vitamin C group the GR enzyme activity was 4% higher as compared to control group. There was significant reduction of GR enzyme activity after gentamicin treatment. In group IV reduction of GR enzyme activity was less marked (Table-1).

Total Antioxidant Status

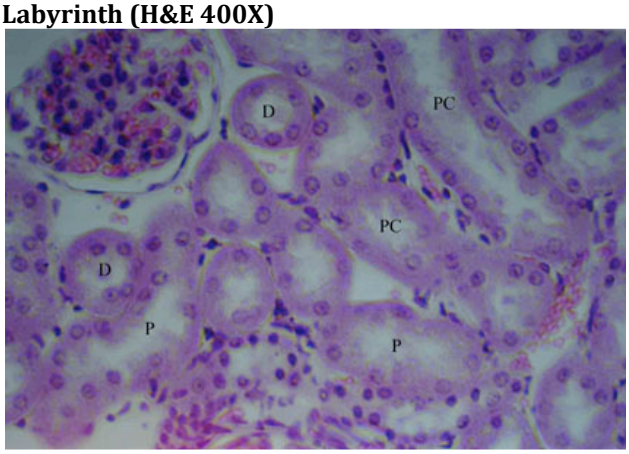
In vitamin C group there was significant increase in TAS level (28%) as compared to the control group. In gentamicin group there was significant reduction in the value of TAS (19%). In group where vitamin C was co-administered the TAS value was higher as compared to gentamicin group (Table-1).

Blood Urea and Serum Creatinine

In gentamicin treated group there was significant rise in blood urea and serum creatinine level. In the group where vitamin C was co-administered the values were significantly higher as compared to gentamicin group (Table-1).

Histopathological Study

Figure-1: Photomicrograph of Vehicle Treated Group I Showing Normal Rat Kidney Cortical Labyrinth (H&E 400X)



Cortical labyrinth includes various proximal tubules (P) and distal tubules (D). The proximal tubules have a slightly larger outside diameter than do the distal tubules. The proximal tubules have a brush border, whereas distal tubules have a cleaner, sharper luminar surface. The lumen of the proximal tubules may be star or S shaped. Typically fewer nuclei appear in the proximal tubule than in an equivalent segment of a distal tubule. Proximal convoluted tubules (PC) are elongated or seen with C-shape. A part of glomerulus is also visible.

The histopathological analysis of kidney from saline treated group revealed the structure of the nephron. The cortical structure along with proximal as well as distal tubules are well organized (Figure-1). In gentamicin treated group the histopathological study revealed disruption of some cortical but mostly tubular

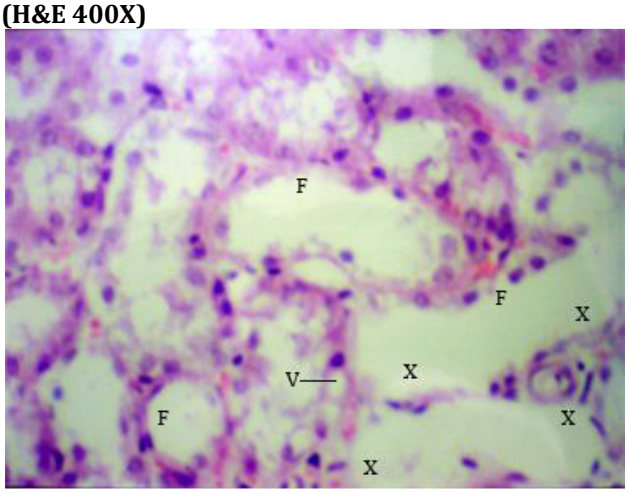
Table-1: Effect of Gentamicin and Vitamin C Induced Changes in Blood Urea, Serum Creatinine, Glutathione Peroxidase (GPx), Glutathione Reductase (GR) and Total Antioxidant Status

Group	Drug Treatment	Blood Urea (mg%)	Serum Creatinine (mg%)	GPx (U/l)	GR (U/l)	Total Antioxidant Status (mmol/l)
I	Saline	38 ± 1.6	0.57 ± 0.13	1010 ± 30	77 ± 7.7	1.28 ± 0.04
II	Vitamin C	40 ± 2.7	0.61 ± 0.14	1104 ± 62	80 ± 4.5	1.64 ± 0.04 ***
III	Gentamicin	51 ± 1.2 ***a	1.11 ± 0.14 *	840 ± 43 *aa	47 ± 1.6 *aa	1.04 ± 0.03 ***aaa
IV	Gentamicin + Vitamin C	46 ± 1.3*	0.59 ± 0.12	921 ± 28 a	85 ± 8.9bb	1.37 ± 0.02aaabbb
ANOVA F ; d P value		10.76 ; 3,20 0.0002	3.851 ;3,20 0.025	7.02 ;3,20 0.0021	7.299 ;3,20 0.0017	54.6 ;3,20 0.0001

Values are mean ± SEM; *P<0.05, **P<0.01 and ***P<0.001 when compared to group I. a P<0.05, aa P<0.01 and aaa P<0.001 when compared to group II. b P <0.05 ,bb P<0.01 and bbb P<0.001 when compared to group III

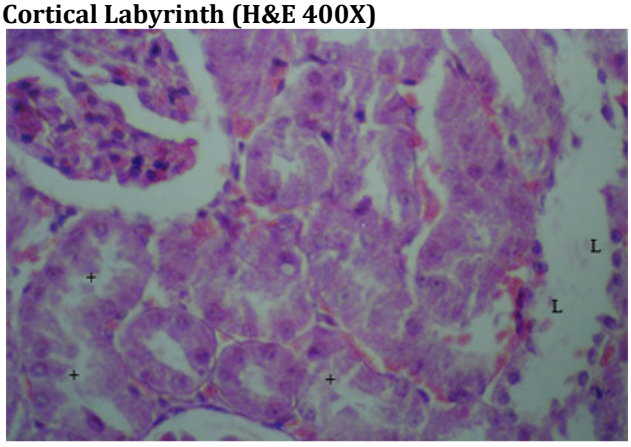
structure. Majority of the tubules are damaged especially proximal convoluted tubules. Vacuolation, necrosis and varying degree of necrosis is seen along with loss of brush-border is evident (Figure-2). Minimal disruption of renal structure was there in group IV. Most cortical parts were intact with some tubular damage (Figure-3).

Figure-2: Photomicrograph of Gentamicin Treated Group II Showing Rat Kidney Cortical Labyrinth (H&E 400X)



Extensive proximal tubular damage appears with disruption of epithelial cells. In one PCT complete disruption of epithelium is seen (X). In some proximal tubules vacuolation (V) appears. Varying degree of necrosis is found in many proximal tubules. Severe damage to brush border is also one of the prominent feature in this picture. Two distal tubules with less degree of damage are seen on outer side of the picture. Flattened epithelium with elongated nuclei (F) is indicative of regenerative process.

Figure-3: Photomicrograph of Gentamicin and Vitamin C Treated Group III Showing Rat Kidney Cortical Labyrinth (H&E 400X)



A large proximal convoluted tubule is seen with extensive damage to brush border and epithelial cell lining (L), but the presence of numerous well-preserved nuclei in the same tubule indicates vitality of the epithelium. Lesser degree of damage with just loss of brush border is apparent in some of the proximal tubules (+). In other tubules brush border is completely intact. The normal architecture is well preserved in many tubules.

DISCUSSION

In the management of gram negative infections, gentamicin has still maintained a leading role, despite the introduction of newer less toxic antimicrobial agents. Gentamicin is water-soluble antibiotic with peak-concentration-dependant bactericidal activity. It is eliminated by glomerular filtration, but a fraction is reabsorbed in the proximal convoluted tubule. There is always higher concentration of gentamicin in proximal tubules of kidneys. Gentamicin demonstrates trough-concentration-dependant nephrotoxicity.

Many mechanisms have been put forward to explain the toxicity of gentamicin, however recently enhanced oxidative stress as a mediator of nephrotoxicity has been suggested and therefore role of antioxidants therapy for prevention of the same has been recommended.[10,11,12]

In the present study renal damage produced by gentamicin was confirmed by histopathological examination of the kidneys. The drug exhibited widespread damage to the kidney architecture. Marked tubular damage was seen especially of proximal tubules. Some of the proximal tubules showed disruption of cells while others showed marked degenerative changes. Certain proximal tubular cells demonstrated varying degree of necrosis. So the main site of gentamicin induced renal damage can be recognized as tubular structure, especially proximal convoluted tubules.

In the recent years increasing interest is seen in the role of free radicals and oxidative damage in a variety of human diseases.[13,14,15] Free radicals are chemical species possessing an unpaired electron that can be considered as fragments of molecules which are generally very reactive.[16] Free radicals have the potential to oxidize biomolecules including proteins, lipids and DNA. The enzyme GPx is selenium dependant enzyme and its main function is removal of H2O2 and it prevents formation of highly reactive hydroxyl (OH-) radical. GR is also an important enzyme

with main function of restoration of cellular glutathione level by reducing oxidized disulfide glutathione GSSG. Total antioxidant status (TAS) study gives a guideline for individual's ability to stand against oxidative stress.

To elucidate the possible involvement of oxidative stress in gentamicin-induced nephrotoxicity we selected GPx, GR and TAS as the markers of oxidative stress. After 30 days of treatment with gentamicin there was significant reduction in the level of GPx, GR and TAS, indicating the increased level of oxidative stress. Sandhya and Varalaxmi[17], using 100 mg/kg/day of gentamicin also found significant reduction in GPx, GSH, SOD and catalase level in rats. Apart from that they also observed an increased production of malondialdehyde (MDA), which is an end product of lipid peroxidation in the kidney. Soejima et al. also found similar correlation while investigating the generation of lipid peroxides and changes in total phospholipids levels in the kidney tissue of rats with gentamicin, at a dose of 80 mg/kg/day. They found persistent decrease in the glutathione peroxidase activity after gentamicin therapy.[18] Kavutcu et al also found significant reduction in the level of GPx while studying effect of gentamicin on renal antioxidant enzyme activity in guinea pigs.[19] Reduction in the level of enzymes like GPx, GR, SOD and catalase after aminoglycoside therapy leads to a predictive reduction in TAS value. The present study confirms generation of oxidative stress in gentamicin induced nephrotoxicity.

Having established that the oxidative stress is induced by gentamicin, we tried to explore the possibility of preventing this by supplementing the antioxidant therapy. For this purpose we selected vitamin C which is a water-soluble antioxidant and free radical scavenger and also freely available in nature.[20, 21, 22] It is plentiful in citrus fruits and is the cheapest antioxidant. It can trap the most notorious hydroxyl radical and it has also capacity to scavenge peroxyl radicals.[23]

The data from present study indicate that the co-administration of vitamin C along with gentamicin showed significant restoration of GPx and GR enzyme activity. Hong et al also observed protection against a potent nephrotoxicant 4-aminophenol, 4-amino-2, 6-diclorophenol (ADCP) by using 0.18 mg/kg i.p. dose of vitamin C.[24] In another study performed by Bradberry and Vale significant protective effect of vitamin C (0.5 mg/kg/day) was observed against chromium-induced nephrotoxicity in rats.[25] In the biological system there seems to be a competition between vitamin C and enzymes GPx and GR for the removal of free radicals. As a result there is less utilization of the antioxidant enzymes. The study of TAS in rats treated with only vitamin C indicated a significant rise in the TAS as compared to control one. This confirms the antioxidant property of vitamin C. The histopathological analysis of the group IV kidney revealed preservation of renal architecture. There was less tubular damage and high power study showed loss of renal brush border without damage to nuclei.

The aminoglycosides are capable of generating free radicals in vitro.[26] Aminoglycoside antibiotics have been shown to enhance the generation of super oxide anion and hydrogen peroxide by renal cortical mitochondria.[27,28,29] The interaction between superoxide anion and hydrogen peroxide in the presence of metal catalyst can lead to the generation of hydroxyl radical. The mitochondrial DNA (mtDNA) is susceptible to oxidative damage by the ever-increasing levels of ROS and free radicals in the mitochondrial matrix. Spillage of ROS into the cytoplasm can further aggravate damage to the various subcellular structures. Free radicals also cause suppression of DNA synthesis leading to loss of cell integrity and protein leakage.[12]

Such in vitro observations have been supported by in vivo studies in which scavengers of reactive oxygen metabolites and iron chelators have been shown to be protective in gentamicin-induced acute renal failure. According to Zima et al ROS can act as second messenger system for the activation of cytokines via NF-kappaB transcription factor; stimulate the formation of TNF-alpha, IL-1, IL-6 and influence the

expression of monocyte-specific cytokines (CSF-1 and MCP-1). Radicals formed in this way can activate proteolytic enzymes (proteinases) which break down collagen and other components of the extracellular matrix present in the basal membrane of glomeruli and in the mesangium.[30]

Observations of the present experimental study indicate a positive correlation of renal damage with reduction in antioxidant enzyme levels and total antioxidant status, thereby generation of oxidative stress. However the predictive value of monitoring these parameters is still suspective since a clearer picture may emerge following more of such detailed studies.

Co-administration of antioxidant substances like vitamin C is expected to prevent and/or reverse the nephrotoxicity caused by gentamicin as seen in the present study. Once again more extensive studies are required to substantiate the benefits of antioxidants in such situations.

CONCLUSION

Renal damage due to gentamicin is associated with oxidative stress. Coadministration of vitamin C along with gentamicin significantly prevents gentamicin induced nephrotoxicity by virtue of its antioxidant effect.

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Cite this article as: Acharya CR, Thakar HN, Vajpeyee SK. A study of oxidative stress in gentamicin induced nephrotoxicity and effect of antioxidant vitamin C in Wistar Rats. Natl J Physiol Pharm Pharmacol 2013; 3:14-20.
Source of Support: Nil
Conflict of interest: None declared