Abstract

We investigated the effect of vitamin C against acute kidney injury (AKI) in female Albino Wister rats. AKI was produced by gentamicin as a simple method for induction AKI, so this achieved by injection of gentamicin as single dose [100mg/kg intra peritoneal (I.P) a day for 10 days] in rats. Vitamin C (200mg/kg I.P a day) was administered 1hr before AKI induction by gentamicin for 10 days.

The blood urea nitrogen (BUN), serum creatinine (Scr) concentrations and Malondialdehyde (MDA) level were markedly elevated in gentamicin-AKI group after treatment 78.05±0.591mg/dl, 1.169±0.017 mg/dl and 85.70±0.587mg/dl (P<0.0001) respectively, but these elevations were significantly suppressed by vitamin C in vitamin C–treated AKI group 17.81±0.457mg/dl, 0.724±0.009 mg/dl and 50.30±0.213 nmol/g (P<0.0001) respectively.

These findings suggest that vitamin C can protect the renal damage caused by gentamicin.

Key words: AKI, gentamicin, vitamin C.

Introduction

Acute kidney injury (AKI) is a relatively common condition in the intensive care unit and occurs in 20% to 30% of critically unwell patients, with approximately 6% eventually requiring renal replacement therapy [1]. The development of AKI in this setting is associated with increased mortality, increased hospital residence, and increased healthcare resource use and costs [2].

Gentamicin (GM) is probably the most commonly used and studied of all the aminoglycosides.[4,5] The limitation to the use of this antibiotic is
its tubular toxicity.[3,4] GM inhibits oxidative phosphorylation and reduces ATP levels in renal tubular cells.[6] Hence, GM-enhanced reactive oxygen species (ROS) formation in isolated cortical mitochondria[6] and ROS-induced cell death were found to have a role in GM-mediated acute renal failure.[3].

Oxidative stress may results in overproduction of oxygen free-radical precursors and/or decreased efficiency of the antioxidant system. The oxygen free-radical generation is associated with auto-oxidation of glucose, decreased glutathione metabolism, alterations in the antioxidant enzymes and formation of lipid peroxides. MDA is used as marker of oxidation of membrane phospholipids through lipid peroxidation [7].

Vitamin C (Vit C) is an antioxidant supplement that exhibits its powerful scavenging effects against activated oxygen species and various free radicals by neutralizing ROS and decreasing oxidative damage to cell membranes. That is why; vitamin C has been used as protective antioxidant agent against numerous kinds of deteriorations caused by oxidative stress [8].

Materials and Methods

Experimental animals.
Female Albino Wister rats (weighing 100-200g, brought from animals house in College of Veterinary Medicine-University of Kufa) were used for these experiments and they were kept at 25 ±2 C °and in a 12hr day-night cycle. They had free access to tap water and commercial chow.

Experimental protocols:
All rats were divided into 4 groups, each one has 10 rats;
Control group: Rats in this group have received 1 mL/day I.P of normal saline for 10 days [9].
Vitamin C group: Rats have received Vitamin C (200mg/kg I.P a day) for 10 days [9].
Vitamin C 500mg x 50 ampules which manufactured by: ATLANTIC laboratories company limited.

Gentamicin-induced AKI group: AKI was produced by injection of gentamicin (100mg/kg I.P a day) [9] for 10 days. Gentamicin was provided from A.MENARINI industrial pharmaceutical company, Florence-Italy.

Vitamin C–treated AKI group: Vitamin C (200mg/kg I.P a day) was administered 1 hour before AKI induction[which was produced by injection of gentamicin (100mg/kg I.P a day)] for 10 days.

To determine the serum parameters, blood samples were obtained from tail vein of rats with sterilized disposable needles before experiment and on the day 11th of experiment, serum creatinine (Scr) were measured by Jaffe method [11] while blood urea nitrogen (BUN) measured by monoxime method [12].

MDA Assay
Malondialdehyde (MDA) level, as an index of lipid peroxidation, was measured chemical assay procedures. MDA, as a thiobarbituric acid reactive substance (TBARS), reacts with thiobarbituric acid (TBA) to produce a red colored complex that has peak absorbance at 532 nm [13].

For this purpose, Phosphoric acid (3 ml; 1%) and TBA (1ml; 0.6%) were added to 0.5 ml of serum in a centrifuge tube and the mixture was heated for 45 min in a boiled water bath. Then when cooled, 4 ml of n-butanol was added to the mixture and vortex-mixed for 1 min followed by centrifugation at 20000 rpm for 20 minutes. The organic layer was transferred to a fresh tube and its absorbance was measured at 520 and 535nm.

Statistical analysis
All data represented as a mean ± S.E.M. Means of groups were compared by one-way analysis of variance (ANOVA) then paired t-test analysis was performed for assessing comparisons before and after experiment. The level of statistical significance was accepted as (P < 0.01). Calculations were performed using the SPSS statistical package (version 17).

**Results**

Figures (1,2 and 3) and table(1) below show the changes of Scr, BUN and MDA concentrations before and after induction of AKI.

Sham operation with or without vitamin C treatment had no detrimental effects before and after experiment, but those in gentamicin group and vitamin C treated gentamicin group were increased after induction.

In the vitamin C treated gentamicin group the elevation of those parameters was smaller than those in the gentamicin group.

The concentrations of BUN decreased markedly to 17.81±0.457mg/dl (P<0.0001) as compared with gentamicin group 78.05±0.591mg/dl (Fig.2& table 1).

Also the concentrations of Scr decreased markedly to 0.724±0.009 mg/dl (P<0.0001) as compared with gentamicin group 1.169±0.017 mg/dl (Fig.1&table 1).

The concentrations of MDA decreased markedly to 50.30±0.213 nmol/g (P<0.0001) as compared with gentamicin group 85.70±0.587mg/dl (Fig.3&table 1).

At the end of this experiment there was no death recorded in all groups. There was no hemoglobinuria or oliguria have been noticed overall experiment.

**Table 1**

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>GROUP</th>
<th>N</th>
<th>BEFORE</th>
<th>AFTER</th>
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<tbody>
<tr>
<td>Blood urea nitrogen mg/dl</td>
<td>Control</td>
<td>10</td>
<td>15.48±0.096</td>
<td>15.40±0.843</td>
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<td>Vitamin C</td>
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<td>15.44±0.060</td>
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<td>Gentamicin induced AKI</td>
<td>10</td>
<td>15.52±0.098</td>
<td>78.05±0.591#¥</td>
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<td>Vitamin C + Gentamicin</td>
<td>10</td>
<td>15.61±0.094</td>
<td>17.81±0.457*€</td>
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<tr>
<td>Serum creatinine mg/dl</td>
<td>Control</td>
<td>10</td>
<td>0.528±0.053</td>
<td>0.524±0.003</td>
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<td>Vitamin C</td>
<td>10</td>
<td>0.533±0.008</td>
<td>0.519±0.004</td>
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<tr>
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<td>Gentamicin induced AKI</td>
<td>10</td>
<td>0.530±0.088</td>
<td>1.169±0.017#¥</td>
</tr>
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<td>Vitamin C + Gentamicin</td>
<td>10</td>
<td>0.535±0.010</td>
<td>0.724±0.009*€</td>
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<tr>
<td>Malondialdehyde nmol/g</td>
<td>Control</td>
<td>10</td>
<td>40.10±0.348</td>
<td>40.20±0.326</td>
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<td>Vitamin C</td>
<td>10</td>
<td>40.90±0.316</td>
<td>40.80±0.133</td>
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<td>10</td>
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<td>85.70±0.587#¥</td>
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<td>Vitamin C + Gentamicin</td>
<td>10</td>
<td>40.30±0.823</td>
<td>50.30±0.213*€</td>
</tr>
</tbody>
</table>

The values represent mean ± S.E.M.

#: P < 0.0001as compared with control group

*#: P <0.0001 as compared with gentamicin group

¥: P <0.0001 as compared with gentamicin group before treatment

€: P <0.001 as compared with vitamin C+ Gentamicin group before treatment
**Discussion**

Aminoglycoside antibiotic GM is commonly used for the treatment of severe gram-negative bacterial infections [14]. However, nephrotoxicity is a major complication of GM administration. Therefore amelioration of nephrotoxicity would enhance its clinical use. Various approaches involving the use of chemical compounds have been used to reduce GM nephrotoxicity [15].
This study demonstrated that gentamicin produced renal damage which is shown as elevated in concentrations of S-CRE and BUN. Moreover, gentamicin increased the level of renal tissue MDA generation, which suggested free radicals involvement in gentamicin-induced nephrotoxicity.

The inhibition of oxygen free radical generation by administration of hydroxyl radical scavenger vitamin C, which provide protection against renal functional impairment after nephrotoxic induced by gentamicin. S-CRE, BUN and MDA concentrations were lowered in rats treated with vitamin C and gentamicin than rats treated gentamicin alone. These results are consistent with results by (Mehmet.et al 2005)(16) whom have reached same results about the role of vitamin C as antioxidant which play important role in nephron-protection that has high importance in clinical applications.

References

Beneficial Effects Of Grape Seed Extract Against Cisplatin-Induced Testicular Damage In Rabbits; Digest Journal of Nanomaterials and Biostructures 2011, Vol. 6, No 1, January-March, p. 155 – 159.


