Vitamin C, an Antioxidant Attenuates Gentamicin-Induced Acute Kidney Injury in Female Albino Wister Rats

Ali Abdil Razzaq Aldallal
Dept. of Basic Sciences, College of Dentistry, University of Kufa, Kufa, Iraq.

Received 26 March 2013 **Accepted 26 June 2013**

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(S*cr*) 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 group17.81±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.

الخلاصة

أجريت هذه الدراسة لبيان تأثير عقار فيتامين س في الحماية من الاصابة الكلوية الحادة المستحدثة بواسطة الجنتامايسين وبتركيز (١٠٠ ملغ/كغم يوميا عن طريق البطن ولمدة ١٠ ايام)عند الجرذان أما عقار فيتامين س فقد أعطي قبل الاستحداث(ساعة واحدة) بواسطة الجنتامايسين و بتركيز (٢٠٠ ملغم/كغم يوميا وعن طريق البطن ولمدة ١٠ أيام. وقد لوحظت التغييرات الحاصلة في مستوى اليوريا نيتروجين والكرياتنين والمالون ثنائي الألديهايد في مصل الدم قبل وبعد الاستحداث التي شهدت ارتفاعا معنويا بعد الاستحداث بواسطة الجنتامايسين على التسلسل التالي :

1.169±0.017 mg/dl ,78.05±0.591mg/dl و (P<0.0001)85.70±0.587 nmol /g .

كما وأظهرت النتائج أن عقار فيتامين س استطاع أن يقلل (بدلالة معنوية) من الارتفاع الحاصل في مستوى اليوريا نيتروجين والكرياتنين والمالون داي ألديهايد في مصل الدم من جراء الاصابة الكلوية الحادة المستحدث بواسطة الجنتامايسين وعلى التسلسل التالي:

(P<0.0001) 50.30±0.213 nmol/g و 0.724±0.009 mg/dl ، 17.81±0.457 mg/dl

وبهذا فان عقار فيتامين س يحمي الكلى من الهبوط في الأداء الوظيفي الحاصل بعد استحداث الاصابة الكلوية الحادة بواسطة الجنتامايسين.

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 (weighing100-200g, 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)[10] 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 11^{th} of experiment, serum creatinine (S*cr)* 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.

Table1 The effect of **vitamin C** on Blood urea nitrogen, Serum creatinine and Malondialdehyde in **Gentamicin** induced – **AKI**

PARAMETER	GROUP	N	BEFORE	AFTER
Blood urea nitrogen mg/dl	**Control**	10	15.48±0.096	15.40±0.843
	Vitamin C	10	15.42±0.100	15.44±0.060
	Gentamicin induced AKI	10	15.52±0.098	78.05±0.591# ¥
	Vitamin C + Gentamicin	10	15.61±0.094	17.81±0.457* €
Serum creatinine mg/dl	**Control**	10	0.528±0.053	0.524±0.003
	Vitamin C	10	0.533±0.008	0.519±0.004
	Gentamicin induced AKI	10	0.530±0.088	1.169±0.017# ¥
	Vitamin C + Gentamicin	10	0.535±0.010	0.724±0.009* €
Malondialdehyde nmol /g	**Control**	10	40.10±0.348	40.20±0.326
	Vitamin C	10	40.90±0.316	40.80±0.133
	Gentamicin induced AKI	10	40.70±0.647	85.70±0.587# ¥
	Vitamin C + Gentamicin	10	40.30±0.823	50.30±0.213*€

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

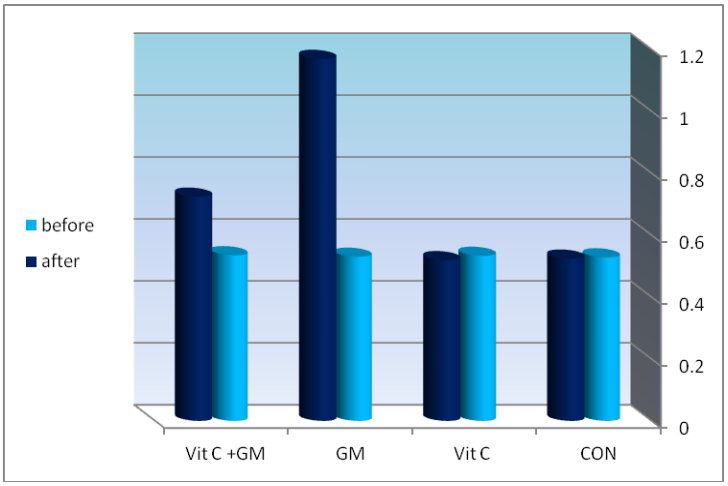

Figure 1 Serum Creatinine mg/dl

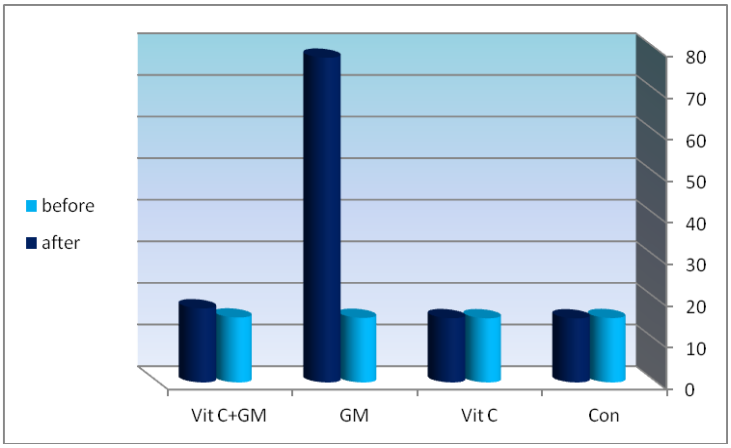

Figure 2 BUN mg/dl

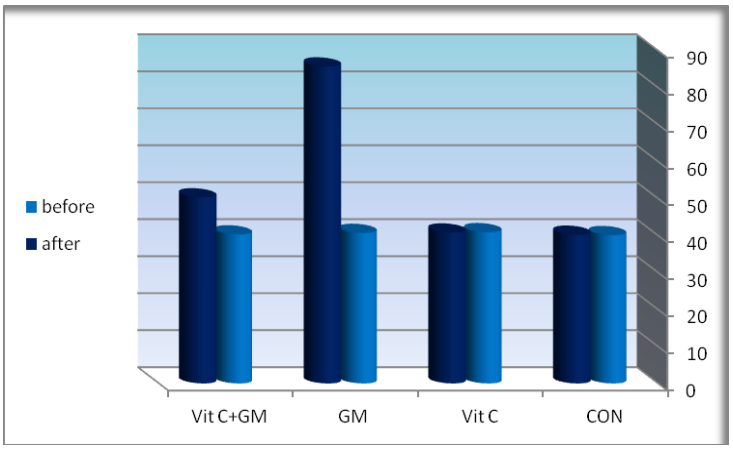

Figure 3 MDA nmol/g

Note: In all figures above show Changes of S-CRE, BUN and MDA before and after gentamicin-induced AKI. Each point represents the mean ± S.E.M.

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

1. Uchino S: The epidemiology of acute renal failure in the world. Curr Opin Crit Care 2006; 12:538–543; Cited by ; Neesh Pannu, MD, SM, FRCP; Mitra K. Nadim, MD (An overview of drug-induced acute kidney injury); Crit Care Med 2008 Vol. 36, No. 4 (Suppl.).

2. Chertow GM, Burdick E, Honour M .Acute kidney injury, mortality, length of stay, and costs in hospitalized patients.J Am Soc Nephrol; ;2005 16:3365–3370; Cited by ; Neesh Pannu, MD, SM, FRCP; Mitra K. Nadim, MD An overview of drug-induced acute kidney injury; Crit Care Med 2008 Vol. 36, No. 4 (Suppl.)

3. Morales AI, Detaille D, Prieto M, Puente A, Briones E, Arévalo M. Metformin prevents experimental gentamicin-induced nephropathy by a mitochondria-dependent pathway). Kidney Int; 201077:861-9. Cited by ;Amini FG, Rafieian-Kopaei M, Nematbakhsh M, Baradaran A, Nasri H. Ameliorative effects of metformin on renal histologic and biochemical alterations of gentamicin-induced renal toxicity in Wistar rats). J Res Med Sci; 2012, 17:621-5.

4. Rafieian-Kopaei M, Nasri H, Nematbakhsh M, Baradaran A, Gheissari A, Rouhi H, Erythropoietin ameliorates genetamycin-induced renal toxicity: A biochemical and histopathological study. J Nephropathol; 2012,1:109-16. Cited by ;Amini FG, Rafieian-Kopaei M, Nematbakhsh M, Baradaran A, Nasri H. Ameliorative effects of metformin on renal histologic and biochemical alterations of gentamicin-induced renal toxicity in Wistar rats. J Res Med Sci; 2012,17:621-5.

5.Lopez-Novoa JM, Quiros Y, Vicente L, Morales AI, Lopez- Hernandez FJ. New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. Kidney Int; 2011, 79:33-45. Cited by ;Amini FG, Rafieian-Kopaei M, Nematbakhsh M, Baradaran A, Nasri H. (Ameliorative effects of metformin on renal histologic and biochemical alterations of gentamicin-induced renal toxicity in Wistar rats. J Res Med Sci; 2012,17:621-5.

6.Kadkhodaee M. Erythropoietin; bright future and new hopes for an old drug. J Nephropathol; 2012,1:81-2. Cited by ;Amini FG, Rafieian-Kopaei M, Nematbakhsh M, Baradaran A, Nasri H. Ameliorative effects of metformin on renal histologic and biochemical alterations of gentamicin-induced renal toxicity in Wistar rats. J Res Med Sci; 2012,17:621-5.

7. A.M. Charissou, C. Cossu-Leguille, and P. Vasseur. Science of The Total Environment, 2004, 322, 109 cited by Nuran Cikcikoglu Yildirim, Fatih Mehmet Kandemir, Fulya Benzer

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

8. Stojiljkovic N, Stoiljkovic M, Randjelovic P, Veljkovic S, Mihailovic D. Cytoprotective effect of vitamin C against gentamicin-induced acute kidney injury in rats. Exp Toxicol Pathol, 2010. Cited by ;Kanwal Rehman, Uzma Saleem, Bashir Ahmed, Ghulam urtaza,Muhammad Sajid Hamid Akash . Nephrocurative And Nephroprotective Effects Of Nigella Sativa Oil In Combination With Vitamin-C In Gentamicin-Induced Renal Toxicity; Indian Journal of Pharmaceutical Science & Research 2012, Vol 2Issue 1pp25-32.

9.Stojiljkovic N, Stoiljkovic M, Mihailovic D, Randjelovic P, Ilic S, Gocmanac-Ignjatovic M, Veljkovic M. Beneficial effects of calcium oral coadministration in gentamicin-induced nephrotoxicity in rats).Ren Fail.; 2012,34(5):622-7.

10. Dur-Zong Hsu, Ya-Hui Li, Pei-Yi Chu, Srinivasan Periasamy, and Ming-Yie Liu. Sesame Oil Prevents Acute Kidney Injury Induced by the Synergistic Action of Aminoglycoside and Iodinated Contrast in Rats;Antimicrobial Agents And Chemotherapy, 2011, June, p. 2532–2536.

11.Chrom V, Rozkosn GK, Sedlk P. Determination of serum creatinine by Jaffe method and how to calibrate to eliminate matrix interference problems.;Clin Chem Lab Med.; 2008, 46(8):1127-33.

12. Robert J.X. Zawada,Peggy Kwan,Kellen L. Olszewski,Manuel Llinas, and Shu-Gui Huang; Quantitative determination of urea concentrations in cell culture medium; Biochem Cell Biol. June ; 2009, 87(3): 541–544.

13. Fernandez J, Perez-Alvarez JA, Fernandezlopez JA.,Thiobarbituric acid test for monitoring lipid oxidation in meat. Food Chem1997, 99,345-353. Cited by Marjan Ajami, Shahriar Eghtesadi, Hamidreza Pazoki-Toroudi,Rouhollah Habibey And Soltan Ahmed Ebrahimi; Effect of crocus sativus on gentamicin induced nephrotoxicity; Biol Res 2010, 43: 83-90.

14. R. J. Reiter, D. X. Tan, R. M. Sainz, J. C. Mayo, and S. Lopez-Burillo, Melatonin: reducing the toxicity and increasing the efficacy of drugs; Journal of Pharmacy and Pharmacology, 2002 , vol.54, no. 10, pp. 1299–1321,. Cited by Pavle Randjelovic,1 Slavimir Veljkovic,1 Nenad Stojiljkovic,Ljubinka Jankovic-Velickovic, Dusan Sokolovic, Milan Stoiljkovic, and Ivan Ilic; Salicylic Acid Attenuates Gentamicin-Induced Nephrotoxicity in Rats. The ScientificWorld Journal Volume 2012, Article, ID 390613, 6 pages.

15. H. Parlakpinar, S. Tasdemir, A. Polat. Protective roleof caffeic acid phenethyl ester (cape) on gentamicin-induced acute renal toxicity in rats;Toxicology, vol. 2005, 207, no. 2, pp. 169–177,. Cited by Pavle Randjelovic,1 Slavimir Veljkovic,1 Nenad Stojiljkovic, Ljubinka Jankovic-Velickovic, Dusan Sokolovic, Milan Stoiljkovic, and Ivan Ilic Salicylic Acid Attenuates Gentamicin-Induced Nephrotoxicity in Rats. The ScientificWorld Journal, Volume 2012, Article ID 390613, 6 pages.

16. Mehmet Kanter, Omer Cosken, Ferah Armutcu, Yesim Hulya and Gulner Kizilay.Protective Effects of VitaminC, Alon or in Combination with VitaminA, on Endotoxin-Induced Oxidative Renal Tissue Damage in Rats;Tohoku J.Exp.Med, 2005, 206,155-162.