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Study of potential nephroprotective effects of Ramipril versus Alpha lipoic acid against Gentamicin induced nephropathy in rats

***Mohamed Y.A. Darwish, **Abd El-Lateef S. Abd El-Lateef**

*Pharmacology Department, Faculty of Dentistry, Sinai University, North Sinai, ** Pharmacology Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt

Corresponding author: **Mohamed Y.A. Darwish,** Phone number: 01553910887, E-mail address: yassinalydarwish@hotmail.com

Abstract:

Background: development of aminoglycosides antibiotics assisted in the cure of infective diseases, Gentamicin (GM) is a well known example that has a wide antibacterial domain.This clinical effect is restricted by the occurrence of kidney functions impairment . **Aim of the work:** we performed this study to examine possible protective effects of Ramipril, Alpha lipoic acid (ALA) and their combinations on kidney functions impairment caused by GM in rats. **Materials and Methods**: fifty male albino rats were categorized into five groups of ten rats. The first group served as control, the second group received GM, the third group received Ramipril and GM, the fourth group received ALA and GM, the fifth group received Ramipril, ALA and GM. Serum biochemical parameters and kidney homogenate oxidative stress parameters were utilized for evaluation of the nephrotoxicity. **Results**: pretreating rats with Ramipril or ALA or their combination attenuated GM induced nephrotoxicity. **Conclusions**: administration of Ramipril alone, ALA alone, or their combination possessed various degrees of nephroprotection against Gentamicin induced nephrotoxicity in rats.

Key words: gentamicin nephrotoxicity, alpha lipoic acid, Ramipril-kidney functions, oxidative stress.

Introduction:

Usage of aminoglycosides antibiotics especially GM is basically hindered due to their nephrotoxicity [1]. GM induced nephropathy has been investigated in many experimental animals as in rabbits, mice and rats [2]. Many approaches as drugs and herbals have been utilized to diminish or reverse renal GM toxicity [3].This would be helpful to augment the safety of the drug and provided that the pharmaceutical manufacturers are not financing sufficiently the development of new antibiotics, with the increasingly arise of resistant strains of microbes, reuse of old antibiotics might be a feasible choice [4] .This particular effect of GM is attributed to its exceptional aggregation in the proximal convoluted tubules of the kidney 50 to 100 times more than serum [5] .One of the principal elements that intermediates GM-induced renal impairment is Reactive Oxygen Species (ROS) [6]. ROS produce cellular injuries and tissue death via several mechanisms including peroxidation of membrane lipids; protein denaturation and Deoxyribonucleic acid (DNA) damage [7]. The scavengers of ROS safeguard the kidney against GM induced nephropathy [8].

A significant number of studies now show that LA and its reduced form, dihydrolipoic acid (DHLA), directly scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS) species and protect cells against a host of insults where oxidative stress is part of the underlying etiolLipoic acid, and dihydrolipoic acid are ROS scavengers; they could guard renal cells against variable insults where oxidative stress is part of disease pathogenesis [9] .

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ALA administration diminishes oxidative injury in the kidney and is correlated with a significant improvement of renal function [10].

Gentamicin alters D-glucose movement through the renal brush border membrane thus confounding energy system of the cells. Alpha lipoic acid (ALA) enhances glucose transporting and increases glycolytic enzymes [11].Another mechanism that leads to GM nephrotoxicity is the reduction of glomerular filtration rate resulting from aggregation of aminoglycosides in the proximal tubular cells leading to inadequacy of renal concentrating power. The utilization of angiotensin converting enzyme inhibitors (ACEIS)

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interferes with the renin angiotensin system (RAS) hindering renal damage, through its action on efferent arteriolar resistance and mesangial cell contractility, also via reducing breakdown of vasodilator kinin and enhancing formation of vasodilator prostaglandin [12].

This experimental study aimed to study possible nephroprotective effects of Ramipril, Alpha lipoic acid (ALA) and their combination on kidney deterioration caused by GM administration to rats.

Materials and Methods:

Drugs and Chemicals:

GM ampoules 40 mg/ml was obtained from Memphis Company for Pharmaceuticals and Chemical Industries, Cairo, Egypt; ALA was obtained from EVA Pharma for Pharmaceuticals and Medical Appliances, Giza, Egypt; 300 mg capsule of Alpha lipoic acid was diluted in 30 ml of distilled water to obtain working dose concentration used in this study [13] . Ramipril was obtained from Sanofi Egypt 10 mg tablet was dissolved in 10 ml of distilled water; Serum Na^+ and K^+ levels were measured using kits supplied by Spectrum Diagnostics (Egyptian Co. for Biotechnology, Oboor City Industrial Area, Cairo, Egypt). Serum blood urea nitrogen (BUN), creatinine were measured using commercial kits supplied by Spinreact (Spinreact, GIRONA, Spain), using a UV spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd, Korea).Lipid Peroxidation (MDA), and SOD Assay Kits were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Animals:

This study was done on 50 male albino rats with average weight 200 gm; they were fed on ordinary diet and tap water in air conditioned animal house. The animals were obtained from Animal House, National Research Centre (Cairo, Egypt). The rats were classified into groups each of 10 rats. The groups of the studied animals and drugs received were shown in table 1. The study was approved by The Ethical Committee of Faculty of Medicine, Al Azhar University,

Table 1: groups of studied animals and given drugs

Group name	Drugs
Group A (control group)	Received 1mL of saline intraperitoneally (i.p.) daily for 8 days.
Group B (GM control group)	Received gentamicin in a single dose of 80 mg/kg by i.p. injection daily for 8 days.[14]
Group C (Ramipril pretreated group)	Received ramipril orally at dosage of 0.9 mg /kg once daily for 18 days,[15] at tenth day Gentamicin was given as group B.
Group D (ALA pretreated group)	Received alpha lipoic acid orally at dosage of 25 mg/kg once daily for18 days,[11] at tenth day Gentamicin was given as group B.
Group E (Alpha lipoic acid and Ramipril pretreated group)	Received alpha lipoic acid and ramipril with gentamicin as in groups B,C, and D.

Regarding all rat's groups at the nineteenth day of the experimental period blood samples were collected from the retro orbital venous plexus under diethyl ether anaesthesia. The following serum biochemical analyses were carried out: serum creatinine level and BUN, Na^+ and K^+. After blood samples collection, finally, all rats were sacrificed and sample of renal tissue of each animal was dissected for estimation of oxidative stress the kidney tissue samples were thawed and homogenized (10% w/v) in 0.15 M KCl at 4 °C then centrifuged at 10000 X g for 90 min. The supernatant were used as the source of experimental product for determination of oxidative stress biomarkers MDA, and SOD.

Statistical analysis

All data were expressed as the mean ± SE. The comparison between groups was done by using One Way Analysis of Variance (ANOVA) test followed by post hoc analysis using LSD (Least Significant Difference) test. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant if less than 0.05.

Results:

Administrating GM has significantly increased serum creatinine, serum blood urea

nitrogen and serum K^+ in comparison to normal control group. In rats treated with GM renal homogenate`s SOD level was significantly decreased wheras MDA level was significantly increased when compared to normal control group as emphasized in Table 2.

Table 2: different biochemical parameters in normal control group versus GM treated group:

	Normal control Mean ± SE	**GM control Mean ± SE**	**P**
Serum Cr. (mg/dl)	0.57 ± 0.06	2.92 ± 0.29	0.000*
Serum BUN (mg/dl)	20.32 ± 1.49	93.58 ± 8.53	0.000*
Serum Na (mEq/L)	151.00 ± 1.05	148.80 ± 0.71	0.232
Serum K (mEq/L)	5.07 ± 0.20	6.24 ± 0.15	0.000*
MDA (µmol/mg ptn)	2.72 ± 0.12	4.14 ± 0.16	0.000*
SOD (µ/mg ptn)	45.30 ± 0.57	39.10 ± 0.45	0.000*

*P value of < 0.05 is considered significant (S). P value of > 0.05 is considered non-significant (NS).

Pretreatment with Ramipril significantly decreased serum Cr, serum BUN, serum Na^+, and serum k^+ when compared to GM control group. Regarding antioxidant parameters in renal homogenate MDA level has significantly decreased as emphasized in table 3.

Table3: different biochemical parameters in GM treated group versus Ramipril pretreated group:

	GM control Mean ± SE	**Ramipril pretreated group Mean ± SE**	**P**
Serum Cr. (mg/dl)	2.92 ± 0.29	1.35 ± 0.02	0.001*
Serum BUN (mg/dl)	93.58 ± 8.53	81.86 ± 1.26	0.015*
Serum Na (mEq/L)	148.80 ± 0.71	143.40 ± 2.09	0.004*
Serum K (mEq/L)	6.24 ± 0.15	4.56 ± 0.17	0.01*
MDA (µmol/mg ptn)	4.14 ± 0.16	3.68 ± 0.13	0.011*
SOD (µ/mg ptn)	39.10 ± 0.45	38.40 ± 0.81	0.564

*P value of < 0.05 is considered significant (S). P value of > 0.05 is considered non-significant (NS).

Prophylactic administering ALA significantly decreased serum Cr, serum BUN, and serum k^+ when compared to GM treated group, with significant decrease in MDA level and increase in SOD level in renal homogenate as emphasized in Table 4.

Table 4: **difference between GM control group versus ALA pretreated group as regarding different biochemical parameters**

	GM control Mean ± SE	**ALA pretreated group Mean ± SE**	**P**
Serum Cr. (mg/dl)	2.92 ± 0.29	0.56 ± 0.03	0.001*
Serum BUN (mg/dl)	93.58 ± 8.53	20.81 ± 1.27	0.001*
Serum Na (mEq/L)	148.80 ± 0.71	149.80 ± 1.14	0.586
Serum K (mEq/L)	6.24 ± 0.15	4.90 ± 0.16	0.01*
MDA (µmol/mg ptn)	4.14 ± 0.16	2.05 ± 0.11	0.01*
SOD (µ/mg ptn)	39.10 ± 0.45	47.46 ± 0.86	0.001*

*P value of < 0.05 is considered significant (S). P value of > 0.05 is considered non-significant (NS).

Prophylactic giving of Ramipril and ALA caused a significant decrease in serum Cr, serum BUN, and serum k^+ when compared to GM control group, also significantly decreased MDA level and increased SOD level as emphasized in table 5.

Table 5: difference between GM control group versus Ramipril+ALA pretreated group as regarding different biochemical parameters:

	GM control Mean ± SE	**ALA + Ramipril pretreated group Mean ± SE**	**P**
Serum Cr. (mg/dl)	2.92 ± 0.29	1.38 ± 0.03	0.01*
Serum BUN (mg/dl)	93.58 ± 8.53	36.26 ± 3.26	0.001*
Serum Na (mEq/L)	148.80 ± 0.71	146.38 ± 1.09	0.189
Serum K (mEq/L)	6.24 ± 0.15	5.00 ± 0.19	0.01*
MDA (µmol/mg ptn)	4.14 ± 0.16	3.66 ± 0.06	0.008*
SOD (µ/mg ptn)	39.10 ± 0.45	39.40 ± 0.81	0.804*

*P value of < 0.05 is considered significant (S). P value of > 0.05 is considered non-significant (NS).

Combining Ramipril with ALA could not significantly enhance its nephroprotective effect except in decreasing serum BUN as emphasized in table 6.

Table 6 : difference between GM control group versus Ramipril pretreated group versus ALA pretreated group versus Ramipril and ALA pretreated group as regarding different biochemical parameters:

	GM control group (Mean ± SE)	Ramipril pretreated group (Mean ± SE)	ALA pretreated group (Mean ± SE)	Ramipril and ALA pretreated group (Mean ± SE)
Serum Cr. (mg/dl)	2.92 ± 0.29	1.35 ± 0.02*	0.56 ± 0.03*†	1.38 ± 0.03*
Serum BUN (mg/dl)	93.58 ± 8.53	81.86 ± 1.26*	20.81 ± 1.27*†	36.26 ± 3.26*†
Serum Na (mEq/L)	148.80 ± 0.71	143.40 ± 2.09*	149.80 ± 1.14	146.38 ± 1.09
Serum K (mEq/L)	6.24 ± 0.15	4.56 ± 0.17*	4.90 ± 0.16*	5.00 ± 0.19*
MDA (µmol/mg ptn)	4.14 ± 0.16	3.68 ± 0.13*	2.05 ± 0.11*†	3.66 ± 0.06*
SOD (µ/mg ptn)	39.10 ± 0.45	38.40 ± 0.81	47.46 ± 0.86*	39.40 ± 0.81

* Significantly different from GM treated group, † Significantly different from Ramipril pretreated group.

Discussion

In our study, we compared the prophylactic effects of Ramipril on GM-induced nephropathy in rats versus those of ALA.

Administration of a single dose of GM 80 mg/kg intraperitoneally daily for 8 days led to a significant rise in serum creatinine, serum BUN and serum potassium when compared to the normal control group.

Our results are similar to study of **Ravindrakumar and Jignesh** [16] who found that in Gentamicin treated group the concentration of renal function parameters like serum urea, blood urea nitrogen and creatinine were considerably elevated as compared to the control group. The renal functional impairment in GM-treated animals can be attributed to reduction in glomerular filtration rate and increased tubular re-absorption of non protein nitrogenous compounds as a result of GM-induced renal damage [17].Variable effects on serum sodium and serum potassium in GM treated rats could be attributed to specific action of aminoglycosides on permeability and transport mechanisms across plasma membrane to these particular ions. Increased urine volume and nephrogenic diabetes insipidus can occur early in aminoglycoside nephrotoxicity [18].As regarding antioxidant parameters in kidney homogenate of GM treated rats in our study we found that there was a significant decrease in SOD level and increase in MDA level in comparison with normal control group. Our results are similar to a study carried out by **Ravindrakumar *et al.*** [16] who found that in kidney homogenate of GM treated animals there was a significant reduction in the levels of protein, superoxide dismutase, reduced glutathione and catalase in comparison with the normal control animals. This effect on antioxidant parameters is likely due to oxidative stress produced in tubular cells on treatment with GM [19].

This oxidative stress is mediated by hydroxyl radicals from hydrogen peroxide and by superoxide anions from mitochondrial origin [20]. Gentamicin directly increased the production of mitochondrial ROS from the respiratory chain [8].Regarding Ramipril usage in GM treated rats it significantly reduced serum Cr, serum BUN, serum Na+ and serum k^+ when compared to GM control group. These results coincide with results of **Attiyah *et al*.** [21] who studied the possible effects of Captopril on GM induced nephrotoxicity in rats and reported that captopril retarded the progression of renal damage, and the concomitant administration of captopril and GM improved the deteriorations in renal functions induced by GM. These protective effects of Ramipril was explained by considering that one of the sequences of events led to nephrotoxicity was failure of glomerular filtration due to decreased renal perfusion pressure or persistent pre-glomerular vasoconstriction, possibly related to the activity of the renin angiotensin system. Ramipril interrupt rennin angiotensin system (RAS) thus reversing those effects [22].Other possible

mechanisms for the action of angiotensin converting enzyme inhibitors which deserve consideration included impairment of the degradation of vasodilator kinin and increased production of vasodilator prostaglandin [12] .

The precise mechanism by which angiotensin converting enzyme inhibitors ($ACEI_S$) reduces GM nephrotoxicity may be due to its anti-inflammatory effect. This anti-inflammatory effect has been linked to amelioration of nuclear factor kappa beta (NF-KB) dependent pro-inflammatory factors that have been linked to increase inflammatory response in many experimental studies [23-25] .The mechanism by which converting enzyme inhibitors mitigate the progression of glomerular injury warrants further investigation their potential effect of the RAS may enhance the understanding of progressive renal disease. In our study, Ramipril administration led to a significant decrease in MDA level in renal homogenate when compared to GM treated rats. Regarding effect on serum sodium; Ramipril usage led to decrease in serum sodium significantly when compared to GM control group unlike ALA. Both drugs significantly decreased serum potassium. Effect on serum sodium may be due to prevention of the conversion of Angiotensin I (ATI) to ATII; thereby decreasing the production and release of aldosterone from the adrenal cortex. This results in an overall reduction in the re-absorption of Na+ and water and allows for the retention of potassium [26].

In our study, potassium was not elevated with the use of Ramipril; this may be attributed to defects in urinary concentrating power and irresponsiveness to antidiuretic hormone (ADH) characterizing tubule-interstitial injury and are mainly due to inability of the affected kidney to maintain the hypertonicity of the medullary interstitium, and inhibition of ADH activity via altering adenylate cyclase action [27].On comparing antioxidant effects of Ramipril versus ALA; SOD level in renal homogenate was significantly increased when treating GM rats with ALA wheras Ramipril usage failed to elevate SOD level in renal homogenate when compared to GM control group. Both drugs when used separately were capable of decreasing MDA level significantly in renal homogenate when compared to GM control group; this suggests more powerful antioxidant properties of ALA than Ramipril.ALA is a potent antioxidant that acts by scavenging oxygen free radicals, redox interaction with other antioxidants, and inhibition of lipid peroxidation [28]. ALA administration markedly minimized inducible Nitric Oxide Synthase (iNOS) expression in renal tissues of GM treated animals [29]. Whereas, non-sulphydryl-containing ACEI as Ramipril inhibited lipid peroxidation only [30].

ALA also diminished renal damage caused by GM as it improved glucose movement and increased levels of glycolytic enzymes which were altered by GM, thus restored energy system efficiency in renal tubular cells [11].Combining Ramipril with ALA could not significantly enhance its nephroprotective effect except in decreasing serum BUN.

Conclusion: prophylactic use of Ramipril successfully attenuated renal damaged caused by Gentamicin. Combining it with Alpha lipoic acid improved its effect against elevated serum BUN.

Conflict of interest:

The authors declare that they have no conflicts of interest.

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