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THE PROTECTIVE EFFECT OF CO-SUPPLEMENTATION OF N-ACETYL CYSTEINE AND MAGNESIUM AGAINST GENTAMICIN INDUCED NEPHROTOXICITY IN ALBINO RATS

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ABSTRACT

Increased production of reactive oxygen species (ROS) may be a common pathway in atherosclerosis, diabetes, cataract, accelerated ageing, and drug toxicities. The protective role of N-acetyl cysteine (NAC) and magnesium (Mg^{+2}) in gentamicin induced nephrotoxicity was evaluated . One hundred albino rats were divided into 5 groups. *Sham control* group (A), *Gentamicin group* (B), *Gentamicin + NAC group* (C), *Gentamicin* + Mg^{+2} *group* (D), and *gentamicin + (NAC* + Mg^{+2}) *group* (E) . Twenty four hour urinary albumin excretion (UAE), plasma creatinine (crP), superoxide dismutase (SOD), catalase (CAT) ,reduced glutathione(GSH) , and malondialdehyde (MDAP) were measured. CrP ,UAE and MDAP were significantly increased in gentamicin treated group, while SOD, CAT , and GSH were significantly decreased compared with those in the control group. Combination between NAC and Mg^{+2} produced a significant decrease of the lipid peroxidation production and increase of the antioxidant enzymes. Increased lipid peroxidation and decreased antioxidant enzymes may play a role in gentamicin induced renal toxicity. Combined NAC + Mg^{+2} may ameliorate these changes and protect the kidney.

KEY WORDS:aminoglycosides-oxidative stress-kidney.

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INTRODUCTION

Aminoglycoside antibiotics including gentamicin are widely used in the treatment of gram-negative infections. Their bactericidal activity is due to their ability to bind with prokaryotic ribosomes and They block the ribosomal formation complex inducing mistranslation,So protein synthesis is inhibited or deranged causing bacterial death [1]. Chronic aminoglycoside use can be complicated by well described nephrotoxic and ototoxic effects [2, 3]. The nephrotoxic potential of gentamicin is well documented in both man and experimental animals[4]. Accumulation of aminoglycosides within the renal cortex is known to be intimately related to the pathogenesis of nephrotoxicity. The specificity of gentamicin for renal toxicity is apparently related to its accumulation in the renal proximal convoluted tubules. The mechanism of toxicity may be related to interactions with the cell membrane, mitochondria, lysosomes and microsomes [5]. Both in vivo and in vitro gentamicin administration to rats depresses the activity of glutathione peroxidase (GPx) and leads to increased the generation of hydroxyl radicals and peroxidation of lipids and suggests that aminoglycoside antibiotics can stimulate the formation of free radicals [6,7]. In addition, ROS scavengers and antioxidants as glutathione and/or vitamin B6 are used to ameliorate gentamicin induced nephrotoxicity [3,8,9]. The major source of oxidative stress is an enzyme reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-derived superoxide anion (O^{2-}) that directly damage cells and activate signaling of all proliferation, adhesion molecules and fibrosis. O^{2-} also inactivates endothelium derived nitric oxide and cause endothelial nitric oxide synthase uncoupling (NOS) [10]. NAPH oxidase is a critical component of both phagocytes and non phagocytes cells including fibroblasts, endothelial cells, renal mesangial cells and tubular cells[11]. N-acetyl cysteine (NAC) is a small molecule containing a thiol group which has potent antioxidant properties and is freely filterable with ready access to intracellular compartments [10]. The diversity of pharmacological applications of NAC is due mainly to the chemical properties of the cysteinyl thiol group[12]. The mechanism of NAC related organ protection is primarily attributed to scavenging ROS either directly by reduced thiol group or indirectly through increasing intracellular glutathione concentrations [13]. In addition to being the precursor of L-cysteine and glutathione reductase, NAC acts as a superoxide scavenger capable of tripling endothelial NOS expression as well as increasing No bioavailability. NAC reacts best with the hydroxyl radical and is poorly reactive with hydrogen peroxide and the superoxide radical. Because of these properties, NAC is widely used in a variety of disease states, as paracetamol toxicity, pulmonary oxygen toxicity, human immunodeficiency virus infection and contrast-medium-induced nephropathy in acute myocardial infarction[14]. Magnesium is the fourth most abundant cation in the body. It is a predominantly intracellular cation and is involved in maintaining ionic cellular balance and enzyme activities as well as in ion channel modulation. Moreover, Mg^{+2} inhibits MDA formation in endothelial cells and low Mg^{+2} induced lipid peroxidation [15]. Thus based on reported properties of Mg^{+2} and NAC, we assumed that either Mg^{+2} supplementation alone or in combination with NAC might have a protective effect against gentamicin induced renal toxicity.

METHODS

Drugs and reagents

Unless stated, all reagents were purchased from Sigma Chemical Co. (St. Louis, USA).

Experimental animals

Experiments were performed according to the *Guide for the Care and Use of Laboratory Animals* (Institute for Laboratory Animal Research, National Research Council, Washington, DC: National Academy Press, no. 85-23, revised 1996). All protocols were approved by our local committee of Animal Care

and Use Committee. One hundred Sprague Dawely rats, 6 weeks old, weighing 150-200g were used and segregated into 5 groups (20 rats each).

Group A: Sham control group: rats were injected intraperitoneally with 5 ml 0.9%.NaCL for 10 successive days.

Group B: Rats were injected intraperitoneally with gentamicin(100 mg/kg/ BW) [3,16] for 10 successive days .

Group C: Rats were given simultaneously gentamicin intraperitoneally+ NAC (440 mg/kg BW orally via gastric tube) for 10 successive days [17] ***.***

Group D: Rats were given MgCL 2.6 H_20 (0.6%)[15]dissolved in drinking water simultaneously with gentamicin intraperitoneally for 10 successive days.

Group E: Rats were given a combination of NAC + Mg^{+2} + gentamicin for 10 successive days.

Animals were maintained on a 12 standard diurnal rhythm cycle and were given food and water ad libitum. 24 hour urine samples were collected in individual metabolic cages for measurement of UAE one day prior to sacrifice.

Sample collection: By the 11th day the animals were anaesthetized using diethyl ether. Blood samples were drawn from the venous plexus deep to the medial canthus of the palpebral fissure. 5 ml blood was added to an anticoagulant (EDTA) centrifuged at 2,000 g for 10 min at 4°C to separate plasma from erythrocytes. The biochemical parameters were determined in plasma : *Creatinine* (modified Jaffe method using creatinine kit, Yeong Dong Pharmaceutical Co.). MDA was measured (modified method of Ohkawa) [18], CAT (Beutler method) [19], *SOD* activity [20]***,*** reduced glutathione (glutathione colorimetric assay kit , Bio diagnostic). *UAE* was measured (rapid colorimetric method , Commercial Kit ,ABC Diagnostic).

Statistical analysis

Data are expressed as mean ± SD. One-way ANOVA test ,followed by Tukey`s post-hoc test was used for data analysis ($p< 0.05$ was considered significant). The statistical analysis of results was done by SPSS (statistical Package for Social Science) program,version 13,2004,for windows XP professional.

P1= Significance versus sham control group
P2= Significance versus gentamicin group

P3= Significance versus NAC+gentamicin group
P4= Significance versus Mg^{+2}+ gentamicin group
Pearson correlation was expressed, $P < 0.05$ was considered significant.

RESULTS

Gentamicin administration produced a significant increase Cr P, and UAE, ($P<0.05$), in comparison to the control group. On the other hand , in comparison to gentamicin group, all the treated groups showed a significant decrease in Cr and UAE, ($P<0.05$, table I). Parameters of lipid peroxidation and antioxidant enzymes are shown in table (II). MDA P in gentamicin group was significantly increased, compared with the control group, while plasma SOD, CAT and reduced glutathione were significantly decreased, ($P<0.05$). The treated groups, showed a significant decrease in MDA P, associated with a significant increase in plasma SOD, CAT and reduced glutathione, in comparison to gentamicin group,($P<0.05$). On the other hand, the NAC + Mg^{+2} combination treated group showed a significant increase in SOD activity and reduced glutathione, in comparison to the gentamycin + NAC and gentamycin + Mg^{+2} treated group alone, $P<0.05$, with no significant difference in MDA and CAT activity in combination group , in comparison to gentamycin + NAC or gentamycin + Mg^{+2} respectively , $P>0.05$, table (II). Correlation analysis(Table III) showed that with the increase of MDA and the decrease of SOD, CAT, reduced glutathione in plasma, the level of UAE was increased

Table I
Effects of gentamicin,gentamicin+ NAC and or+ Mg^{+2} on Cr P and UAE

Group	*Plasma Cr mg/dl*	*UAE* mg/24 h
Control	0.67±0.07	18.3±3.4
Gentamicin.	1.49±0.37	64.4±9.8
P1	<0.05	<0.05
Gentamicin+NAC	1.02±0.19	29.7±4.3
P2	<0.05	<0.05
Gentamicin +Mg^{+2}	1.10±0.16	32.5±5.6
P2	<0.05	<0.05
Gentamicin + NAC + Mg^{+2}	0.83±0.10	21.5±3.90
P1	Non sig	Non sig
P2	<0.05	<0.05
P3	<0.05	<0.05
P4	<0.05	<0.05

Data are expressed as mean ± SD(20 rats/group).
One-way ANOVA test with Tukey`s post-hoc test (p< 0.05 significant(sig).
P1= versus control group.
P2= versus gentamicin group
P3= versus NAC+gentamicin group
P4= versus Mg^{+2} +gentamicin group

Table II
Effects of gentamicin, gentamicin+ NAC and or+ Mg^{+2} on plasma MDA, CAT ,SOD and GSH

Group	MDA nmol/l	CAT activity umol/ml min	SOD 0/0inhibition	GSH nmol/ml
Control	5.17±1.10	1.56±0.18	19.01±2.56	3.02±0.30
Gentamicin	8.9±1.60	1.07±0.20	10.7±3.36	1.73±0.25
P1	<0.05	<0.05	<0.05	<0.05
Gentamicin +NAC	5.35±0.77	1.34±0.30	16.58±2.40	2.65±0.50
P2	<0.05	<0.05	<0.05	<0.05
Gentamicin+ Mg^{+2}	5.83±1.17	1.30±0.36	14.6±3.66	2.45±0.31
P2	<0.05	<0.05	<0.05	<0.05
Gentamicin +NAC+ Mg^{+2}	5.23±0.50	1.4 ±0.32	17.4±3.90	2.86±0.52
P1	Non sig	Non sig	Non sig	Non sig
P2	<0.05	<0.05	<0.05	<0.05
P3	Non sig	Non sig	<0.05	<0.05
P4	Non sig	Non sig	<0.05	<0.05

Data are expressed as mean ± SD(20 rats/group).
One-way Anova test with Tukey`s post-hoc test (p< 0.05 significant).
P1= versus control group.
P2= versus gentamicin group
P3=. versusNAC+gentamicin group
P4= versus Mg^{+2}+gentamicin group

Table III
Correlations between the studied parameters

		Cr	UAE	MDA	CAT	SOD	GSH
Cr	Pearson Correlation	1	.724**	.587**	-.365-**	-.546-**	-.515-**
	Sig.		.001	. 001	. 001	.001	.001
	N	100	100	100	100	100	100
UAE	Pearson Correlation	.724**	1	.761**	-.450-**	-.613-**	-.708-**
	Sig.	.001		.001	.001	.001	.001
	N	100	100	100	100	100	100
MDA	Pearson Correlation	.587**	.761**	1	-.415-**	-.457-**	-.569-**
	Sig.	.001	.001		.001	.001	.001
	N	100	100	100	100	100	100
CAT	Pearson Correlation	-.365-**	-.450-**	-.415-**	1	.313**	.262**
	Sig.	.001	.001	.001		.001	.009
	N	100	100	100	100	100	100
SOD	Pearson Correlation	-.546-**	-.613-**	-.457-**	.313**	1	.434**
	Sig.	.001	.001	.001	.001		.001
	N	100	100	100	100	100	100
GSH	Pearson Correlation	-.515-**	-.708-**	-.569-**	.262**	.434**	1
	Sig.	.001	.001	.001	.009	.001	
	N	100	100	100	100	100	100

Pearson Correlation was expressed, between MDA , SOD, CAT, glutathione and UAE.
P-value <0.05 was considered significant (sig).

DISCUSSION

Aminoglycoside antibiotic, gentamicin can produce nephrotoxicity in human Proximal tubular cells which are a major site of damage in patients treated with gentamicin [21]. The results of this work indicate that gentamicin induced nephrotoxicity which affects the proximal convoluted position of the renal tubules and to a less degree the glomeruli and the distal convoluted tubules,gentamicin accumulates in the renal cortex due to the rich blood supply and its reabsorption in the proximal convoluted tubules which receives most of the blood perfusing the kidney causing degeneration and necrosis of the epithelial cells of these tubules. The specificity of gentamicin for renal toxicity is related to it is concentrated in the renal proximal convoluted tubules[3]. The adverse interaction of the drugs with one or more critical intracellular processes leads to renal cortical phospholipidosis and disruption of functions of membranes and organelles including mitochondria and microsomes. It has been reported that in vivo and in vitro gentamicin administration to rats depresses the activity of glutathione peroxidase and leads to increased generation of hydroxyl radicals , reactive oxygen species (ROS) and peroxidation of lipids by altering mitochondria [6,22]. Some studies demonstrated that antioxidants administration have ameliorated gentamicin induced nephropathy. [21] A role for superoxides in gentamicin mediated nephropathy is suggested when different superoxide dismutase treatments were shown to be effective in ameliorating renal injury in gentamicin induced nephrotoxicity[7]. In another

study lipid peroxide levels were reduced and levels of an antioxidant enzymes and thiol compounds were increased following administration of α-tocopherol and ascorbic acid in lead induced oxidative stress[22]. In the present study, the role of ROS in gentamicin induced nephrotoxicity was assessed by administration of NAC and Mg^{+2}. In the present work, there was a decrease in MDA with an increase in SOD, CAT and glutathione concentration in gentamicin treated with NAC and Mg^{+2}. This probably occurs in part by scavenging the very reactive O^2-otland Roo by Mg^{+2} and NAC[23,24].

GSH provides a major protection in oxidative injury by involving in the cellular defense system against oxidative damage[25]. Reports indicate that tissue injury induced by various stimuli is coupled with GSH depletion[26,27]. This tripeptide is present in high amount in the kidney. GSH scavengers O^2-and protects protein thiol groups from oxidation. It also has a major role in restoring other free radical scavengers and antioxidants such as α-tocopherol and ascorbic acid to their reduced state [28]. The low GSH levels in the present study agree with previous reports and may be due to its consumption during oxidative stress[29]. There was also an increase in plasma GSH in gentamicin groups treated with NAC . NAC may exert its antioxidant effect by facilitating GSH biosynthesis and supplying GSH for GSH peroxidase-catalyzed reactions [30]. NAC protects kidney tissue against oxidative damage may be through a direct reaction with hydroxyl radicals and also as a source of sulfhydryl groups[27] . Much of great interest about microalbuminuria derives from the fact that albumin excretion is a risk factor for kidney failure [31] and it reflects glomerular dysfunction[32]. A greater increase in (UAE) was observed in gentamicin group than in groups treated with NAC and Mg^{+2}. Thus indicates tubular and glomerular fibrogenesis, moreover, an association between microalbuminuria and Mg^{+2} depletion has been reported[33]. There was a decrease in (UAE) in Mg^{+2} and NAC treated groups. This provides a support for the effect of Mg^{+2} and NAC in attenuation of microalbuminuria that may be linked to their antioxidant activity and inhibition of lipid peroxidation[34]. *Lastly ,the combined NAC+ Mg^{+2} treated gentamicin group also showed a normalization in the anti oxidative parameters ,plasma creatinine and* correlation analysis indicates that with the increase of MDA and the decrease of SOD, CAT, glutathione in plasma, the level of UAE was increased which implies that increased lipid peroxidation and decreased antioxidant enzymes in plasma may play a role in gentamicin toxicity[7,9,34].

CONCLUSION

It could be concluded that gentamicin induced nephrotoxicity can be ameliorated effectively by the simultaneous administration of NAC and Mg^{+2}.

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- *Conflict of interest*

There are No actual or potential conflicts of interest.

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