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Nephrotoxicity and Oxidative Stress of Single Large Dose or Two Divided Doses of Gentamicin in Rats

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Abstract: Gentamicin (GS) is a potent antimicrobial exhibiting concentration dependent bacterial killing. A high dose of gentamicin (10 mg kg^{-1}) is required to reach sufficient concentrations in specific fluids as cerebrospinal fluid and to be effective on antibiotics resistant bacteria as well as treatment of acute and dangerous illness. Using a rat model, the renal toxicity and oxidative stress of administering gentamicin (10 mg kg^{-1} daily for 7 days) either in a single dose or divided into 2 doses was investigated. The safety of dose regimens was assessed through oxidant-antioxidant parameters as well as renal function tests. Typical renal damage and high oxidative stress were evident in the control group receiving 100 mg kg^{-1} gentamicin daily for 7 days. This was verified by high serum urea, uric acid, creatinine as well as increase in the levels of oxidative stress biomarkers as malondialdehyde, NO, total antioxidant capacity and decrease in reduced glutathione level. At any of the used regimen, 10 mg kg^{-1} gentamicin did not provide high compromise for renal functions nor significantly increased the oxidative stress and tissue damage. Based on microscopic lesions scores and biochemical analysis, there were no significant differences between single or two divided dosages of gentamicin at dose rate of 10 mg kg^{-1} day^{-1}. Further studies are required for applications in other animals of human subjects.

Key words: Gentamicin, nephrotoxicity, renal function, oxidative stress, drug toxicology, posology

INTRODUCTION

Gentamicin (GS) is a potent antimicrobial acting on gram negative and some gram positive micro-organisms of human and animal infections (Ouedraogo *et al.*, 2008; Martinez *et al.*, 2010; Yong *et al.*, 2010). However, the use of gentamicin is associated with various toxic effects, especially nephrotoxicity. The nephrotoxic effect of gentamicin was previously established (Nourani *et al.*, 2006; Martinez-Salgado *et al.*, 2007; Balakumar *et al.*, 2010; Dehghani *et al.*, 2011). Furthermore, the amelioration of aminoglycosides induced nephrotoxicity was extensively studied (Adeneye and Benebo, 2008; Abdel-Raheem *et al.*, 2009; Balakumar *et al.*, 2010; Bibu *et al.*, 2010; Salem *et al.*, 2010; Heeba, 2011; Abdelaziz and Kandeel, 2011). The nephrotoxicity of aminoglycosides was associated with renal tubular tissue damage which is associated with the production of reactive oxygen species (Abdelaziz and Kandeel, 2011).

Gentamicin exhibits concentration dependent bacterial killing, in which high concentration of drug is the best way to get rapid bactericidal effect (Hagen and Oymar, 2009). Thus, for pharmacokinetic and pharmacodynamic aspects, administration of higher doses of the drug is more preferable than smaller doses. However, there is no accurate data about their corresponding toxicities. Extending dosing intervals yields a longer period at a lower drug concentration, thereby producing less renal toxicity (Kiatchoosakun *et al.*, 2005). Furthermore, there is still insufficient evidence from current available data to finally state that one of the dosing regimens is better than the other (Rao *et al.*, 2006).

The usual dosage of gentamicin is ranging from 2-5 mg kg^{-1} b.wt., however, in critically ill patients and dangerous infections, the dose could be increased up to 7.5-10 mg kg^{-1}. In order to get adequate therapeutic efficacy in critically ill septic patients, the initial dose of gentamicin could be increased above 7 mg kg^{-1}

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(Pereira *et al.*, 2009). In brucellosis, gentamicin 7 mg kg^{-1} day^{-1} was highly effective (Ertem *et al.*, 2000), while 7.5 mg kg^{-1} day^{-1} was given in acute pneumonia (Dekate *et al.*, 2011). Furthermore, a high dose of gentamicin (10 mg kg^{-1}) is required to reach sufficient concentrations in specific fluids as cerebrospinal fluid and to be effective on antibiotics resistant bacteria (Tateda *et al.*, 1999; Cottagnoud *et al.*, 2002). In the majority of gentamicin studies, the dosage regimens were testing 2-5 mg kg^{-1} gentamicin dosages (Roushan *et al.*, 2010). However, in cases of severe illness and certain types of infections higher doses of gentamicin could be given. Data comparing such higher dosages of gentamicin is scarce. In this respect, no reports describe the effects of gentamicin at dose rate of 10 mg kg^{-1}.

In the present study, the effect of two different administration systems was compared using a rat model of gentamicin-induced nephrotoxicity. The administration of 10 mg kg^{-1} gentamicin once daily or divided into 2 doses of 5 mg kg^{-1} twice daily for 7 days was investigated. The renal damage parameters as well as the markers of oxidative damage were used to assess the safety of the used administration methods.

MATERIALS AND METHODS

Drugs and chemicals: Gentamicin was obtained from Memphis Company for pharmaceutical and chemical industries. All other chemicals and reagents are purchased were of the best quality available.

Animals: Twenty adult Wistar-albino rats weighting approximately 180-200 g were used in this experimental study. Rats were housed in stainless steel cages and received feed and water *ad-libitum*. All experiments in this study were performed in accordance with the guidelines for animal research from the National Institutes of Health and were approved by our local Animal ethics committee.

Experimental design: Rats were divided randomly into four equal groups including five animals each: (1) GS group: 100 mg kg^{-1} GS was injected intraperitoneal (i.p.) to rats for 7 days. (2) One dose GS group (1GS): 10 mg kg^{-1} GS was injected i.p., to rats for 7 days. (3) Two doses GS group (2 GS): 5 mg kg^{-1} GS was injected i.p., to rats twice daily for 7 days. (4) Control group: The rats received a daily i.p., injection of 0.5 mL isotonic saline for 7 days. The rats were sacrificed on the eighth day; blood samples were collected and let to clot at room temperature and centrifuged. Fresh blood as well as serum samples was subjected to biochemical analyses.

Biochemical analysis
Determination of BUNBUN level: was measured using Urea Enzymatic Colorimetric Kit (Biosystems, Barcelona, Spain) as described before Young (1997).

Determination of serum creatinine level: Serum creatinine level was determined using Creatinine Colorimetric Kit (Human, Germany) according to the method of Bartels and Bohmer (1971).

Determination of serum uric acid: Serum uric acid level was determined using uric acid Colorimetric Kit (Human, Germany) according to the method of Fossati *et al.* (1980).

Determination of oxidative stress biomarkers: Commercial kits were used to determine the biomarkers of oxidative stress. Reduced glutathione (GSH), Nitric oxide assay and total antioxidant capacity were determined by the spectrophotometric methods.

Lipoperoxidation: Lipoperoxidation was measured by using a commercial kit to determine the amount of malondialdehyde (MDA). MDA reacts with thiobarbituric acid to give a pink reactive product measured at 534 mM.

Histopathological examinations and microscopic lesion scoring: Kidneys of rats were used for histopathological evaluation. Kidney sections were fixed in 10% neutral buffered formaldehyde solution, dehydrated in graded alcohol and embedded in paraffin. Paraffin embedded specimens were cut into 5 μm thickness and stained hematoxylin-eosin for light microscopic examination. All sections of kidney samples were examined for mononuclear cell infiltration, glomerular hypercellularity, necrosis of tubular cells and tubular vacuolation and degeneration. For each slide, minimum of 20 fields (20x)/slide were examined, evaluated and an average score was obtained. The severity of changes is scored according to the following scale: no change (0), mild,<10% tubular damage (1), moderate changes affecting 10-25% of tubules (2), severe (3) damage affecting 25-50% of tubules and extensive damage affecting >50% of tubules (4).

Statistical analysis: All data are presented as Means±SD. Results were evaluated by a one-way ANOVA and significant differences defined with Tukey post-tests. Differences are considered significant when p<0.05. The test parameters were analyzed by analysis of variance by using SPSS 10.0 for Windows.

RESULTS

In the present study, drug-induced nephrotoxicities were established by intraperitoneal injection of a nephrotoxin (gentamicin). These toxicities were characterized by marked biochemical and pathological changes. These changes were associated in marked changes in oxidative stress biomarkers and kidney function tests.

Renal function tests: The effects of aminoglycoside antibiotics on urea, uric acid and creatinine are presented in Table 1. The level of urea and uric acid in GS group was significantly higher than saline, 1 GS and 2 GS groups. While, in 1GS and 2GS groups the levels of urea and uric were significantly lower than that of the GS group and not different from the saline group. There was significant increase in serum level of creatinine (1.9) of GS group ($p > 0.05$) compared with saline group (0.7). 1GS and 2GS groups showed more or less similar levels to saline group.

The markers of oxidative stress: The effects of aminoglycoside antibiotics on biomarkers of oxidative stress are presented in Table 2. The level of GSH was significantly lowered in GS (27.13 mg dL^{-1}) group compared with the saline group (32.16 mg dL^{-1}) ($p < 0.05$). 1 GS and 2 GS groups did not show statistically significant change compared with GS and saline groups. A significant increase in the total antioxidant activity was noticed in GS (0.19 mM L^{-1}), 1 GS (0.17 mM L^{-1}) and 2 GS (0.17 mM L^{-1}) compared with the saline group (0.09 mM L^{-1}). The GS group showed significant increase in MDA activity (80.62 nmol mL^{-1}) compared with saline group (50.98 nmol mL^{-1}), furthermore, 1 GS and 2 GS groups showed significant decrease in MDA levels compared with GS group and did not show significant difference in comparison with the saline group. A small increase in NO level was reported with GS group (4.56 µmol L^{-1}) compared with the saline group (4.09 µmol L^{-1}).

Microscopic lesion scoring: Semiquantitative analysis of cellular infiltration, golmerularhypercellularity, necrosis of tubular cells and tubular cellular damage was adopted to assess the lesions severity using stained sections of kidneys. These changes are summarized in Table 3 and Fig. 1-5. Histopathologic examination of kidney showed marked damage in GS treated rats. GS group showed the highest scores of the measured parameters that were significantly different from the saline group.

The regular findings in renal damaged samples include vacuolation, hydrophobic generation, cloudy

Table 1: Kidney function tests for GS (gentamicin 100 mg kg⁻¹ for 7 days), 1 GS (gentamicin 10 mg kg⁻¹ once daily for 7 days), 2 GS (gentamicin 5 mg kg⁻¹ twice daily for 7 days

Group	Treatment	Urea	Uric acid	Creatinine
1	GS	60.5±7.9³	4.70±0.75³	1.9225±0.2³
2	1GS	33.0±5.9ᶜ	3.80±0.65ᵃ	0.72±0.17
3	2GS	28.2±7.4ᶜ	3.30±0.37ᵇ	0.68±0.04ᵃ
4	Control nontreated	32.0±4.7ᶜ	3.04±0.35ᶜ	0.776±0.09

Significance: ᵃp<0.05 versus gentamicin group; p<0.01 versus gentamicin group; ᶜp<0.001 versus gentamicin group; ³p<0.001 versus saline group

Table 2: Biomarkers of oxidative stress for GS (gentamicin 100 mg kg⁻¹ for 7 days), 1 GS (gentamicin 10 mg kg⁻¹ once daily for 7 days), 2 GS (gentamicin 5 mg kg⁻¹ twice daily for 8 days)

Group	Treatment	GSH (mg dL⁻¹)	Total antioxidant (mM L⁻¹)	NO (µmol L⁻¹)	MDA (nmol mL⁻¹)
1	GS	27.13±3.57¹	0.19±0.01³	4.56±0.74	80.62±6.47¹
2	1GS	30.23±2.35	0.17±0.01³	3.80±0.52	42.88±10.9ᶜ
3	2GS	30.85±3.85	0.17±0.01³	4.32±1.22	41.61±21.97ᵇ
4	Control nontreated	32.16±2.97	0.09±0.01ᶜ	4.09±1.29	50.98±31.7

ᵃSignificance: ᵃp<0.05 versus gentamicin group; ᵇp<0.01 versus gentamicin group; ᶜp<0.001 versus gentamicin group; ¹p<0.05 versus saline group; ³p<0.001 versus saline group

Table 3: Semiquantitative analysis of cellular infiltration, golmerularhypercellularity, necrosis of tubular cells and tubular cellular damage

Group	Treatment	CI	hC	N	TD
1	GS	1.8³	2.5³	1.1	2.6³
2	1GS	0.6ᶜ	0.3ᶜ	0.5	1.3ᵇ
3	2GS	0.9ᶜ	0.6ᶜ	0.1ᵃ	1.1ᵇ
4	Control nontreated	0.9ᶜ	0.1ᶜ	0.2ᵃ	0.8

ᶜCI: Cellular infiltration; hC: Golmerularhypercellularity; N: Necrosis of tubular cells, TD: Tubular cellular damage. Significance: ᵃp<0.05 versus gentamicin group; ᵇp<0.01 versus gentamicin group; ᶜp<0.001 versus gentamicin group; ³p<0.001 versus saline group

swelling, desquamation and necrosis were observed in epithelial cells of the proximal tubules in rats of GS group (Fig. 1-5). In the glomeruli, there was congestion and swelling (Fig. 2). Furthermore, severe inflammatory infiltrate in the form of mononuclear cells either intertubular, periglomerular or perivascular cellular infiltration. The cellular infiltration was either focal aggregation, random or diffuses infiltration of cells (Fig. 3-4). In some other cases, cystic luminal dilatation was evident (Fig. 4).

The scores of histopathological investigations were summarized in Table 3. GS group showed the highest necrotic changes eliciting a score of 1.1 (approximately 10-12% of the examined tubules showed necrotic changes). Furthermore, the tubular cellular damage was involving about 40% of the examined tubules (lesion score of 2.6). 1 GS and 2 GS groups were more or less similar to the saline group and were significantly different from the GS group. Interestingly, results showed that no significant difference between 1 GS and 2 GS groups.
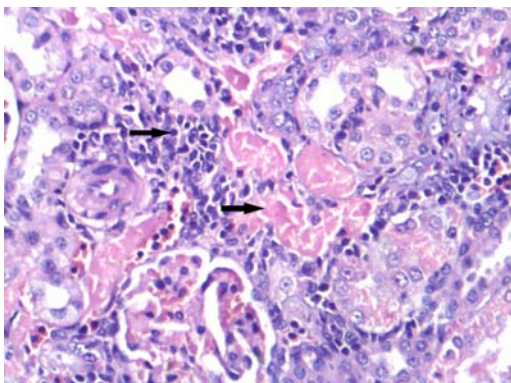
Fig. 1: Histopathological view of renal sections in GS group showing degeneration, desquamation of tubular cells, focal necrosis and cellular infiltration (arrows; H.E.x400)
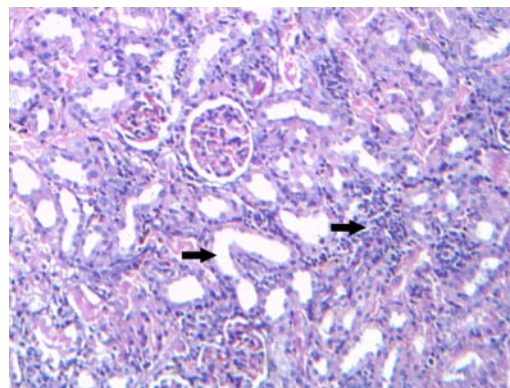

Fig. 2: Histopathological view of renal sections in GS group showing congestion of glomeruli (arrows; H.E.x400)

Fig. 3: Histopathological view of renal sections in GS group showing diffuse mononuclear cell infiltration (arrows; H.E.x400)

Fig. 4: Histopathological view of renal sections in GS group showing cystic dilatation of tubules, focal necrosis and mononuclear cell infiltration (arrows; H.E.x200)
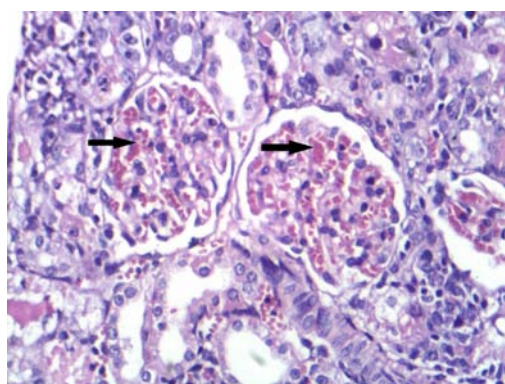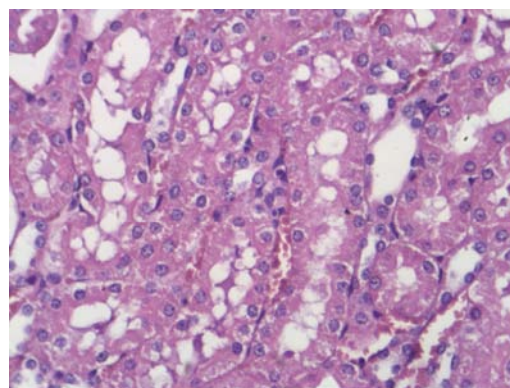

Fig. 5: Histopathological view of renal sections in GS group showing vacuolation and degeneration of tubular cells (arrows; H.E.x400)
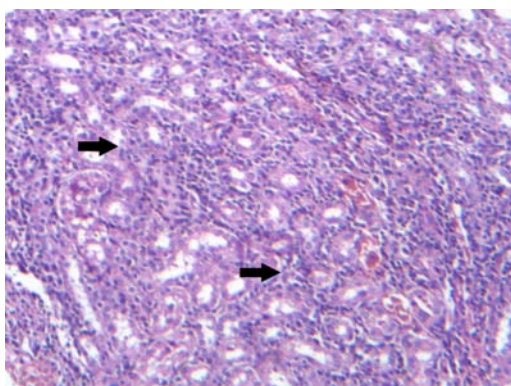
DISCUSSION

The use of aminoglycosides is complicated with its probable nephrotoxicity (Dwivedi *et al.*, 2009). GS is the widely used aminoglycoside which is conserved for severe infections with Gram negative bacteria. The dose rate of 2-5 mg kg^{-1} is recommended for gentamicin. However, in severe infections and those infections in specialized sites needs higher doses of gentamicin. Most previous studies were describing the pharmacokinetics, efficacy and toxicity of lower dosages of gentamicin. However, no reports describe the effects of gentamicin at dose rate of 10 mg kg^{-1} regarding to the administration pattern as well as its effect on renal functions and

oxidative stress. In this work, the administration of 10 mg kg^{-1} gentamicin once daily or divided into 2 doses of 5 mg kg^{-1} twice daily for 7 days was investigated. Furthermore, we compared each administration method regarding to its effect on renal tissues as well as oxidative stress. Oxygen-free radical was found to be an important mediator in GS induced renal damage (Priuska and Schacht, 1995; Yang *et al.*, 1995; Khanahmadi *et al.*, 2010). Aminoglycosides are often given two or three times daily. Recently, increasing numbers of experimental and clinical studies have demonstrated that a once daily dosing regimen may be at least as effective as and possibly less toxic than, multiple dosing.

In this study, the increased level of creatinine, urea and uric acid in GS group indicates renal damage. While, in 1 GS and 2 GS groups the levels of urea and uric were significantly lower than that of the GS group. The evidence of renal damage in GS group was demonstrated in several studies (Abdel-Raheem *et al.*, 2009; Yaman and Balikci, 2010).

GSH protects the cell against the toxic effects of hydroxyl radicals and singlet oxygen. The decrease in GSH level is associated with lower activity of glutathione related enzymes. Hence, more tissue damage due to poor control on free radicals. In this regard, the low level of GSH in GS group indicates more tissue damage. GSH stores were significantly depleted, indicating the use of GSH as an antioxidant for the detoxification of toxic oxygen metabolites (Sirag, 2009). In 1 GS and 2 GS groups, the level of GSH was comparable to the saline group, indicating nonsignificant levels of tissue damage. These results are coinciding with the higher levels of MDA in GS group. MDA is a marker of lipoperoxidation and indicated free radical formation in gentamicin-induced renal toxicity. In this context, the lipoperoxidation is increased during renal damage (Adaramoye, 2009). The lipoperoxidation was significantly decreased in 1 GS and 2 GS groups, indicating lower tissue damage due to lower production of free radicals which are the forerunners for MDA (Uboh *et al.*, 2011).

The biochemical findings were further confirmed by histopathological examination. Where, the GS treated group showed the highest lesions scores which is coinciding with the highest tissue damage. This could be due to the formation of highly reactive radicals as a consequence of oxidative stress caused by GS (Suresh *et al.*, 2008). All these changes were histopathologically reduced in 1 GS and 2 GS groups. Based on lesions scores, there were no significant differences between 1 GS and 2 GS groups, indicating that there was no difference between once or twice daily

dosages of gentamicin at dose rate of 10 mg kg^{-1} day^{-1}. Indeed, gentamicin is eliminated via glomerular filtration but a fraction is reabsorbed in the proximal tubule. Renal uptake of gentamicin is following a saturation phenomenon, wherein the accumulation is less when administered in one large dose than several small doses. In explanation of our finding, we assume that gentamicin at 5 mg kg^{-1} saturates the renal reuptake mechanism that allows similar toxicities of one large dose of 10 mg kg^{-1} or two divided doses of 5 mg kg^{-1}.

CONCLUSIONS

The results strongly suggest that gentamicin dose can be increased to 10 mg kg^{-1} for 7 days. Furthermore, we did not notice significant differences between single administrations of once daily dose or two divided doses of gentamicin at this dose rate. Further studies are required to establish pharmacokinetics and pharmacodynamics of these dose regimens in other subjects.

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