Gentamicin Nephrotoxicity: Animal Experimental Correlate with Human Pharmacovigilance Outcome

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Background: National Agency for Food and Drugs Administration and Control (NAFDAC), which is responsible for pharmacovigilance activity in Nigeria, recently withdrew injection gentamicin 280 mg, used in the management of life-threatening and multidrug-resistant infections from circulation, due to reported toxicity. Thus, this study aimed to investigate the toxicity profile of the commonly used strengths (80 mg and 280 mg) of gentamicin on kidney using animal models.

Methods: Animals were divided into five groups of 16 rats each. For rats of groups 1 and 2, gentamicin (1.14 mg/kg each group) was administered intramuscularly twice daily for 7 and 14 days, respectively, after which eight of them were sacrificed by cervical dislocation. Blood was collected via cardiac puncture and the kidneys were carefully removed and weighed immediately. The remaining eight animals were kept for reversibility study for another 7 and 14 days, respectively. For groups 3 and 4, gentamicin (4 mg/kg each group) was administered as a single daily dose for 7 and 14 days, respectively, and eight animals from the groups were subjected to reversibility study for 7 and 14 days, respectively. Group 5, the control group animals, were given 10 ml/kg distilled water for 14 days. Histopathology of the kidneys, serum creatinine levels, and antioxidant enzyme activities were investigated.

Results: Significant increase ($p \leq 0.001$) in the level of creatinine of rats administered 4.0 mg/kg for 14 days was observed compared with all other groups. Significant ($p \leq 0.001$) elevations in the lipid peroxidation in all gentamicin-administered animals and acute tubular necrosis in most of the gentamicin-administered animals were observed.

Conclusion: Toxicity profile of gentamicin on the kidneys is dependent on both dose and duration of administration. The findings justify the decision made by NAFDAC to ban the use of high-dose inj. gentamicin 280 mg in Nigeria.

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Key words: dose, duration of therapy, gentamicin, nephrotoxicity, reversibility

At a Glance Commentary

Scientific background of the subject

Gentamicin is an aminoglycoside used in the treatment of gram-negative organisms but nephrotoxicity has been a major limiting factor in its therapeutic use. This factor imposes limitations on the total dose as well as the total length of the treatment and particularly requires considerable reduction in the dose in patients with compromised or impaired renal function. Nephrotoxicity has been traced to be due to marked accumulation and retention of aminoglycosides in the proximal convoluted tubules.

What this study adds to the field

It is elucidated from this study that the mechanism of Gentamicin nephrotoxicity is linked to lipid peroxidation. Furthermore, the toxicity profile of Gentamicin on the kidneys is both dose and duration of administration dependent. These findings obtained from this study corroborate the decision by the drug regulatory authority in Nigeria (NAFDAC) to ban the sale, distribution and use of Gentamicin 280 mg strength.

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The use of aminoglycosides is known to be associated with acute kidney injury due to acute tubular necrosis, with a rise in the serum creatinine concentration of more than 0.5-1 mg/dl (44-88 μmol/L) or a 50% increase in serum creatinine concentration from baseline occurring in 10-20% of patients.[1,2] This class of antibiotics also has the potential to cause ototoxicity. Some studies indicate a genetic predisposition to aminoglycoside auditory ototoxicity due to a mutation of mitochondrial deoxyribonucleic acid (DNA).[3,4] However, the aminoglycosides have good efficacy in the treatment of serious gram-negative systemic infections.[5-8] Gentamicin, an aminoglycoside, has been documented to play a significant role in the management of life-threatening and multidrug-resistant infections, particularly when used in combination therapy.[9-11] Administration of geldanamycin, an antibiotic, was recently demonstrated to protect inner ear cells from the toxicity caused by gentamicin.[12] Nevertheless, gentamicin toxicity is the most common single known cause of bilateral vestibulopathy, accounting for 15-50% of all cases[13] and has been known to cause renal cell death by generation of free radicals, phospholipidosis, extracellular calcium-sensing receptor stimulation and energetic catastrophe, reduced renal blood flow, and inflammation.[14]

Conventionally, gentamicin is administered in multiple daily doses. Previous clinical trials have, however, demonstrated equal or better therapeutic response and reduced toxicity by utilizing a larger single daily dosage regimen.[9,10,15] The findings of Munckhof *et al.*,[16] Hatala *et al.*,[17] Ferriols-Lisart and Alos-Alminana;[18] Barza *et al.*,[19] and Prins *et al.*,[20] have all corroborated the fact that administering aminoglycoside once a day is an effective antimicrobial regimen with less nephrotoxicity than the conventional, divided dose regimen. These studies showed that adherence to the protocol for administration and monitoring of gentamicin therapy (Hartford nomogram) improves the clinical efficacy of gentamicin and significantly reduces the incidence of drug toxicity.

Contrary to the larger single daily dosage regimen protocol for the administration of gentamicin as highlighted by the previous studies, the National Agency for Food and Drug Administration and Control (NAFDAC), which controls pharmacovigilance activities in Nigeria, has recently taken a decision that led to the deregistration and subsequent withdrawal of gentamicin 280 mg injection from circulation in Nigeria. This regulatory action was due to recent safety data associating the use of high-dose, single-unit gentamicin injection with ototoxicity, nephrotoxicity, and increased incidence of endotoxin reactions (anaphylactic shock, hemorrhage, fibrinolysis, hypotension, inflammation, vascular coagulation). However, the lower strengths of gentamicin injection are not affected by this withdrawal. Duly registered gentamicin 10 mg, 40 mg, 80 mg, and other approved lower strengths of the injection are available for clinical use in Nigeria.[21]

In view of this, we investigated the toxicity profile of the commonly used strengths (80 mg and 280 mg) of gentamicin on the kidneys using animal models. We also investigated the possible reversibility of the renal damage in line with the report of Bussolati *et al.*,[22] which showed the role of stem cells derived from the bone marrow migration through circulation to the kidney in kidney tissue repair. The findings of the study will thus scientifically be correlated with the NAFDAC pharmacovigilance outcome.

METHODS

Drugs

The injections of gentamicin (80 mg and 280 mg) were obtained from a registered pharmacy outlet in Lagos, Nigeria.

Animals

Albino rats (average weight 130 g) were obtained from Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria. The animals were authenticated in the Zoology Department, Faculty of Science, University of Lagos. They were made to acclimatize for 2 weeks before the commencement of the experiment. The animals were fed on Pfizer animal feed cubes and water *ad libitum*. The investigation conforms to The Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) for studies involving experimental animals.

Experimental procedures

The animals were divided into five experimental groups of 16 animals per group except experimental group 5 (control) that had eight animals. The animals were weighed before the commencement of the experiment and weekly throughout the course of the experiment.

Animals in groups 1 and 2 received gentamicin (1.14 mg/kg each group) intramuscularly twice daily for 7 and 14 days, respectively, after which eight of the animals were anesthetized using diethylether and sacrificed by cervical dislocation. The inhalation anesthetic was applied by placing each animal in a closed receptacle containing cotton wool soaked with diethylether, and thereafter, cervical dislocation was done. Blood was thereafter collected via cardiac puncture, and the kidneys were carefully removed after dissecting the animals and weighed immediately. The remaining eight animals were kept for reversibility study for another 7 and 14 days, respectively. During the period for reversibility studies, the animals were administered feed

and water only. For the animals in groups 3 and 4 respectively, gentamicin (4 mg/kg each group) was administered as a single daily dose for 7 and 14 days, respectively. The remaining eight animals in these two groups were kept for reversibility study for another 7 and 14 days, respectively. Group 5 animals, the controls, had 10 ml/kg distilled water for 14 days.

Other physical pathological observations were carried out to assess the possible physical internal damage.

Biochemical assays

Serum creatinine was determined using fully automated clinical chemistry analyzer (Hitachi 912, Boehringer Mannheim, Mannheim, Germany). Measurement of the activity of kidney antioxidant enzymes and malondialdehyde (MDA) levels was done according to standard procedures. Catalase (CAT; EC 1.11),[19,22-24] MDA (EC 202-974-4),[24,25] superoxide dismutase (SOD; EC 1.15.1.1),[24] and reduced glutathione (GSH; EC 2.5.1.18)[22,23] were measured.

Histopathology

After sacrificing the experimental animals, the sample kidneys of all the animals were fixed in 10% formalin in labeled bottles. Tissues were processed routinely and embedded in paraffin wax. Sections of 5 μm thickness were cut, stained with hematoxylin and eosin, and examined under the light microscope by a pathologist.

Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). The data were subjected to one-way analysis of variance (ANOVA) test followed by Tukey's multiple comparison test using GraphPad Prism 5 (GraphPad Software Inc., San Diego, California, USA). Results were considered to be significant at $p \leq 0.05$.

RESULTS

The survival rate of the animals was 100% during the experimental procedures, and there were no obvious toxic signs observed during the drug administration procedures and dissection of the animals.

The results presented in Table 1 show no significant difference ($p \leq 0.05$) in the weights of both the left and right kidneys across groups after the administration of injection gentamicin (1.14 mg/kg equivalent to 80 mg/70 kg and 4.0 mg/kg equivalent to 280 mg/70 kg) and conducting the reversibility studies.

The results presented in Table 2 show a significant decrease ($p \leq 0.001$) in the levels of SOD, CAT, and vit C in group 1A rats compared with controls and group 4A rats (except vit C). However, there was an increase ($p \leq 0.001$) in the level of MDA compared with controls.

The results showed a significant decrease ($p \leq 0.001$) in the levels of GSH, SOD, CAT, and vit C and a corresponding increase ($p \leq 0.001$) in the level of MDA in rats of group 2A compared with control animals. There was also a significant decrease ($p \leq 0.001$) in the levels of CAT and MDA and an increase in the level of vit C in group 2A animals compared with group 4A animals.

There was a significant decrease ($p \leq 0.001$) in the level of CAT in group 3A rats compared with controls and group 4A rats. Also, a decrease in the level of MDA compared with group 4A rats was observed. Group 3A rats further showed an increase ($p \leq 0.001$) in the level of SOD compared with controls and group 4A rats. However, there was a decrease in the level of vit C compared with that of controls.

It was observed that among the rats in group 4A, there was a decrease ($p \leq 0.001$) in the levels of SOD, CAT, glutathione-*S*-transferase (GST), and vit C, but an increase in MDA level compared with the control animals. There was also a significant decrease in GSH and GST levels and an increase ($p \leq 0.001$) in MDA level compared with the treated rats in group 4B.

The results presented in Figure 1 show statistically significant decrease ($p \leq 0.001$) in the levels of creatinine in the animals of groups 1A, 2A, and 3A compared with the animals of group 4A. There was also a significant increase ($p \leq 0.001$) in the levels of creatinine in the animals of group 4A as compared with the animals of group 4B and controls.

The histopathologic examination of the kidney showed normal architecture of the kidney in treated rats of groups 1A

Table 1: Weight of the kidneys per 100 body weight of rats

	Left kidney/100 g	Right kidney/100 g
Control	0.31±0.01	0.31±0.01
1A	0.28±0.01	0.29±0.01
1B	0.31±0.01	0.29±0.01
2A	0.27±0.00	0.27±0.01
2B	0.29±0.01	0.29±0.01
3A	0.29±0.00	0.31±0.00
3B	0.29±0.01	0.29±0.01
4A	0.27±0.01	0.29±0.01
4B	0.32±0.01	0.32±0.02

The groupings are as follows. Control: Animals administered with distilled water (10 ml/kg); 1A: Animals administered with 1.14 mg/kg inj. gentamicin for 7 days; 1B: Animals kept for 7 days reversibility study after treatment as in 1A; 2A: Animals administered with 1.14 mg/kg inj. gentamicin for 14 days; 2B: Animals kept for 14 days reversibility study after treatment as in 2A; 3A: Animals administered with 4.0 mg/kg inj. gentamicin for 7 days; 3B: Animals kept for 7 days reversibility study after the treatment as in 3A; 4A: Animals administered with 4.0 mg/kg inj. gentamicin for 14 days; 4B: Animals kept for 14 days reversibility study after treatment as in 4A

Table 2: Lipid peroxidation and antioxidants' levels of rats administered with various doses of inj. gentamicin

Group	GSH	SOD	CAT	GST	MDA	Vit C
Control	0.59±0.04	12.00±0.06	81.00±0.41	107.00±5.8	0.02±0.00	41.00±0.49
1A	0.41±0.02	4.50±0.03[ab]	30.00±0.06[ab]	67.00±2.90	0.09±0.00[a]	26.00±0.28[ab]
1B	0.50±0.04	4.90±0.12	33.00±0.78	86.00±4.80	0.09±0.00	31.00±0.53
2A	0.17±0.00[a]	5.50±0.00[a]	37.00±0.03[ab]	36.00±0.90	0.07±0.01[ab]	27.00±0.09[ab]
2B	0.52±0.04	11.00±0.43	76.00±2.90	78.00±8.70	0.10±0.01	27.00±0.28
3A	0.35±0.08	56.00±0.01[ab]	37.00±0.13[ab]	77.00±15.00	0.06±0.00[ab]	22.00±0.49[a]
3B	0.54±0.09	9.40±0.14	63.00±0.92	77.00±11.00	0.09±0.00	24.00±0.24
4A	0.23±0.09[c]	9.50±0.27[a]	63.00±1.80[ac]	28.00±11.00[ac]	0.12±0.00[ac]	22.00±0.69[a]
4B	0.71±0.15	7.70±0.41	52.00±2.70	118.00±29.00	0.05±0.00	25.00±0.94

Abbreviations: SOD: Superoxide dismutase (µmol/mg); CAT: Catalase (µmol/mg); MDA: Malondialdehyde (µmol/mg); GSH: Reduced glutathione (µmol/mg); GST: Glutathione-*S*-transferase (µmol/mg); Vit C: Vitamin C (mg/100 g); [a]$p \leq 0.001$ in comparison with control; [b]$p \leq 0.001$ in comparison with 4A; [c]$p \leq 0.001$ in comparison with 4B. The groupings are as follows. Control: animals administered with distilled water (10 ml/kg); 1A: Animals administered with 1.14 mg/kg inj. gentamicin for 7 days; 1B: Animals kept for 7 days reversibility study after treatment as in 1A; 2A: Animals administered with 1.14 mg/kg inj. gentamicin for 14 days; 2B: Animals kept for 14 days reversibility study after treatment as in 2A; 3A: Animals administered with 4.0 mg/kg inj. gentamicin for 7 days; 3B: Animals kept for 7 days reversibility study after the treatment as in 3A; 4A: Animals administered with 4.0 mg/kg inj. gentamicin for 14 days; 4B: Animals kept for 14 days reversibility study after treatment as in 4A

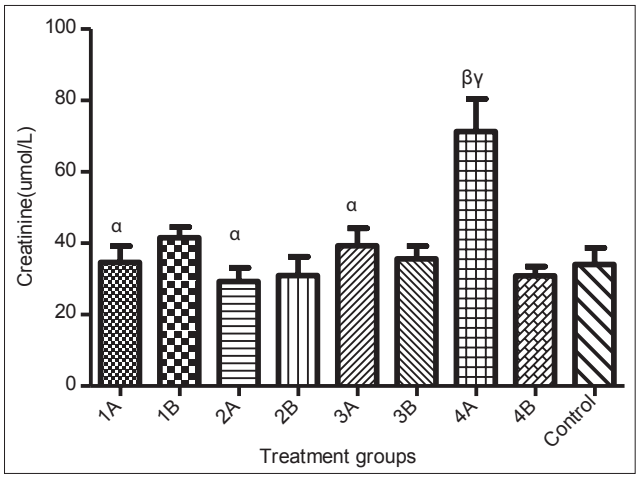

Figure 1: Creatinine levels of rats administered various doses of inj. gentamicin; α, $p \leq 0.001$ in comparison with 4A; β, $p \leq 0.001$ in comparison with 4B; γ, $p \leq 0.001$ in comparison with control animals. The groupings are as follows. Control: Animals administered with distilled water (10 ml/kg); 1A: Animals administered with 1.14 mg/kg inj. gentamicin for 7 days; 1B: Animals kept for 7 days reversibility study after treatment as in 1A; 2A: Animals administered with 1.14 mg/kg inj. gentamicin for 14 days; 2B: Animals kept for 14 days reversibility study after treatment as in 2A; 3A: Animals administered with 4.0 mg/kg inj. gentamicin for 7 days; 3B: Animals kept for 7 days reversibility study after the treatment as in 3A; 4A: Animals administered with 4.0 mg/kg inj. gentamicin for 14 days; 4B: Animals kept for 14 days reversibility study after treatment as in 4A.

and 1B. The treated animals in groups 2A and 2B showed acute tubular necrosis with chronic inflammatory cells. The histopathologic results of group 3A rats seem to be relatively normal with normal kidney architecture, while group 3B rats showed acute tubular necrosis with interstitial nephritis. The results of the histopathologic examinations of rats in groups 4A and 4B showed interstitial fibrosis along with acute tubular necrosis and nephritis.

DISCUSSION

Gentamicin remains one of the most commonly used antibiotics. It is inexpensive and highly effective against gram-negative bacilli. However, the major drawback to gentamicin usage is its narrow margin of safety.[26] The nephrotoxic effect of gentamicin has been well documented, but the recent understanding is that adequate peak serum gentamicin concentration (SGC) and low trough SGC are required to achieve good bactericidal activity and reduce potential toxicity.[27-29] The high peak SGC may be generally achieved with large once daily dose (ODD) gentamicin administration. Thus, the need to substitute the conventional divided doses of gentamicin with ODD is advocated.[16,17,19,20]

The recent pharmacovigilance report by NAFDAC which controls the pharmacovigilance activity in Nigeria has shown that gentamicin administration at a high dose (280 mg) once daily is highly ototoxic and nephrotoxic and is associated with increased incidence of endotoxin reactions (anaphylactic shock, hemorrhage, fibrinolysis, hypotension, inflammation, vascular coagulation, etc.). Activation of the renin–angiotensin system (RAS) is associated with renal fibrosis and progression of renal failure.[30] These adverse reactions have led to the decision of deregistration and withdrawal of this product from use in Nigeria.

The findings obtained from the present study have shown that 280 mg of gentamicin administered once daily increased the serum creatinine levels of the experimental animals: Administration of 280 mg once daily for 2 weeks significantly ($p \leq 0.001$) increased the creatinine levels of experimental animals compared with the controls and administration of 80 mg of gentamicin as twice daily dose, although the reversibility study (280 mg) showed no significant difference in the creatinine levels of the animals after 2 weeks of no drug administration compared with the

controls. This observed reversibility in the creatinine level is not consistent with the histopathologic observations of the kidneys. The histology results showed acute tubular necrosis of the kidneys in the group administered 80 mg dose of gentamicin. Acute tubular necrosis and interstitial fibrosis were observed in both the group administered 280 mg of gentamicin daily for 2 weeks and its reversibility study experimental group. These alterations in the normal architecture of the kidneys could affirm the damage caused by gentamicin to the renal cells. The acute tubular necrosis that was accompanied with interstitial necrosis in the rats given 280 mg of gentamicin once daily (2 weeks) calls for a close attention and reconsideration on whether to continue the inclusion of this strength of gentamicin in official treatment guideline. The observed inconsistent results in the creatinine level and the histopathologic findings of the rats administered 280 mg gentamicin daily for 2 weeks may be due to the body physiology adjusting to normalize the biochemical functions via upregulation of the endogenous antioxidant enzymes, thus normalizing the level of creatinine. However, the gross damage caused by the drug to the renal cells is still visible. There were no marked differences in the weights of both the right and left kidneys across the experimental groups.

The significant ($p \leq 0.001$) increase in lipid peroxidation and decrease in the levels of GSH, CAT, and vit C of all the gentamicin-administered experimental animals compared with the control group animals is an indication that the mechanism of toxicity caused by gentamicin is via free radical generation. This observation is consistent with the study of Du and Yang[31] who showed that gentamicin increases superoxide anion and hydroxyl radical production in renal cortical mitochondria.

Conclusion

Inference may be made from this study that the toxicity profile of gentamicin is dependent on both dose and length of administration. Thus, while using gentamicin, caution must be exercised with the dose administered and the duration of administration. There must be regular therapeutic monitoring of the plasma concentration and elimination rate of gentamicin, and when the use of gentamicin is absolutely inevitable, it may be advisable to incorporate exogenous antioxidants to possibly modulate the free radicals that may be generated by gentamicin. In conclusion, the results obtained show that 280 mg of gentamicin injection administered once daily for 14 days demonstrated high toxicity profile and corroborated the observed nephrotoxicity in human beings, which led to the withdrawal of this strength from the Nigerian market. However, further study may be done with the use of body surface area (BSA) normalization method for extrapolation of animal dose to human equivalent dose. This procedure may translate 4 mg/kg in rats to 0.64 mg/kg in rats.

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