Research Article

Artesunate Affects Diurnal Variation of Gentamicin Nephrotoxicity in Wistar Rats

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ABSTRACT

Objective: Diurnal variation in gentamicin nephrotoxicity has been previously demonstrated. This study investigated the effect of diurnal variation on gentamicin nephrotoxicity with concurrent artesunate administration in wistar rats. **Methodology:** Gentamicin (120 $mgkg^{-1}$) was co-administered with artesunate (100 $mgkg^{-1}$) at 0000 hrs and 1200 hrs being times of least and greatest gentamicin-nephrotoxicity. Renal biomarkers including creatinine, urea, CAT, SOD, MDA, GPx and electrolytes were determined following seven-day co-administration. **Findings:** Gentamicin at 1200 hrs produced significant ($p<0.05$) elevation in serum urea and creatinine in comparison with controls. Animals that received gentamicin at 0000 hrs had significantly lower creatinine and urea levels compared with the 1200 hrs gentamicin group. Artesunate ameliorated gentamicin-nephrotoxicity at both time points with reduction in serum urea and creatinine values. **Conclusion:** The study showed that artesunate ameliorated gentamicin-induced nephrotoxicity during both periods in rats. **Research Value:** This research suggests that the concurrent administration of both drugs in bacteremia and parasitemia co-infection may offer beneficial effects of alleviating gentamicin induced nephrotoxicity irrespective of rest or activity time administration.

Key words: Artesunate, Gentamicin, Nephrotoxicity, Diurnal Variation, Chronotoxicity.

INTRODUCTION

Malaria is a parasitic infection caused by Plasmodium species, and falciparum malaria has been linked with severe complications and mortality.[1] The disease may present in a severe form resulting in death particularly in non immune patients, when diagnosis and treatment are not prompt at the acute phase.[2] Although many countries have been able to decrease their malaria burden significantly between 2000 and 2010,[3] this is not a global success and the treatment and prevention of malaria has continued to undergo reviews and changes in strategies. Current therapy uses artemisinin derivatives in combination with other agents, resulting in rapid clearance, however monotherapy is also deployed for longer duration.[4] Combination regimens typically combine an artemisinin derivative with a long acting agent,[5] while life threatening severe malaria is treated with parenteral artesunate.[6] Artesunate is largely reported to be safe, although, available data in the literature report conflicting effects on the kidney. These include non association with electrolyte imbalance,[7] diuretic effect in malaria patients,[8,9] and renal involvement.[10,11] Recent data suggests that invasive bacterial infection appears to be associated with severe malaria in children,[12] while previous data reported non typhoidal Salmonella with severe malaria in children,[13-15] the therapy of which often includes gentamicin. Gentamicin is used for treatment of sensitive gram negative bacteria, and its nephrotoxicity[16] limits its clinical use.[17] However its use continues because of efficacy, cost, post antibiotic effect and its synergistic effects with other antimicrobials.[18] The strategies to reduce its toxicity include reduction of dosing frequency, consideration of the circadian rhythm of renal

Received Date : 07/04/2016
Revised Date : 27/06/2016
Accepted Date : 29/06/2016

DOI: 10.5530/rjps.2016.2.5

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function,[19] as well as concurrent administration with antioxidants, and modification of time of administration based on temporal variation in its nephrotoxicity. With concurrent administration of gentamicin and artesunate being a clinical situation in bacteria-falciparum infections, the effect of the concurrent drug therapy on the kidney is important. The study thus investigated the effect of chronomodulated gentamicin-artesunate regimens on nephrotoxicity in wistar rats. Serum biomarkers of nephrotoxicity and relative kidney weights were determined following a seven day concurrent administration of artesunate and gentamicin. The effect of the concurrent drug administration on some markers of oxidative stress was also investigated.

MATERIALS AND METHODS

Animals

Male wistar rats with average weight of 180 g were obtained from the Animal Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria Nigeria. The animals were allowed to acclimatize in their new animal room for about one week before experimental procedures began. The rats were housed in cages of five or six and had wood shavings as beddings which were changed frequently. The animals were placed on Vital Feed and public water supply *ad libitum*. Experiments were performed in accordance with approved institutional Animal Committee guidelines (DAC/IW-OT/66-14).

Experimental design

Animals were divided into six groups for the study with the first group serving as saline control. The second group received 120 $mgkg^{-1}$ gentamicin, while the third group received artesunate 100 $mgkg^{-1}$ both at 1200 hrs daily for seven days. Group four received both artesunate and gentamicin also at 1200 hrs daily for seven days. Animals in group five received gentamicin alone at 0000 hrs (midnight) daily for seven days while animals in group six received both gentamicin and artesunate at 0000 hrs similarly for seven days. The 120 $mgkg^{-1}$ dose of gentamicin was adopted from a previously reported protocol,[20] and after an initial pilot study. The dose of artesunate was also based on its established antiplasmodial activity within this dose range.

Preparation of drugs

The drugs used in the study were gentamicin (Gentalek® injection) and artesunate powder. They were obtained via Lek and Tuyil Pharmaceuticals, Nigeria respectively. The drugs were freshly prepared daily for administration via the intraperitoneal route using suitable syringes and needles.

Serum analysis

At the end of the seven consecutive days of daily treatment, the animals were euthanized with chloroform and the kidneys were excised after dissecting the rats. Kidney weights were determined using a sensitive weighing balance. Blood was also collected from the jugular veins of the rats into anticoagulant free vacutainers. Serum urea, creatinine, electrolytes (sodium, chloride, bicarbonate, potassium) AST, ALT, ALP, total protein, albumin and glucose were determined using a Bayer Automated Analyzer and appropriate kits. Serum oxidative stress markers (GPx, SOD, CAT and MDA) were also determined using Randox® kits.

Data analysis

Data obtained was analyzed using One Way Analysis of Variance followed by Levene's test of equality of variance. Welch Robust test of means was used when significant outcome of Levene's test was obtained. The Hochberg post hoc test was used and a p value of ≤ 0.05 was considered statistically significant for each comparison.

RESULTS

Results from the study show that serum urea and creatinine levels of the group that received gentamicin alone at 1200 hrs was significantly ($p=0.007$) higher than that of the saline treated group. However the creatinine levels in all the other groups that received artesunate or gentamicin alone, or a combination of gentamicin with artesunate at all time points were significantly lower than those of animals that received gentamicin alone at 1200 hrs. A similar result was also obtained for the urea levels (Figures 1 and 2). The serum electrolyte levels were not significantly altered by the different times of administration and combinations with gentamicin and artesunate (Table 1). There was also no significant difference in the group that received artesunate alone when compared with the saline group (Table 1). Of the three markers of hepatic functions (AST, ALT and ALP) only the levels of the ALP for animals TREATED WITH GENTAMICIN at 1200 hrs was significantly higher than that of the normal saline control (Table 2). Data from the study did not show any significant difference in any of the markers of oxidative stress that were investigated (Table 3). Serum levels of total protein, bilirubin and glucose also did not differ significantly (Table 4). The relative left kidney weight of all the groups did not differ significantly from the saline group. However, the group that received artesunate alone at 1200 hrs showed significantly lower relative kidney

Table 1: Effect of chronomodulated gentamicin-artesunate administration on serum electrolytes in wistar rats

	Electrolyte levels in mmol/l			
Group	**Sodium**	**Potassium**	**Chloride**	**Bicarbonate**
Normal Saline 1200 hrs	139.00±0.84	4.28±0.07	98.71±1.30	22.42±0.75
Gent 1200 hrs	139.28±0.89	4.18±0.24	99.00±0.72	23.28±1.08
Art 1200 hrs	137.57±0.78	4.04±0.12	96.85±0.70	22.57±1.10
Art+Gent 1200 hrs	138.00±1.09	4.08±0.18	98.57±1.61	22.42±1.47
Gent+Art 0000 hrs	140.50±1.25	4.20±0.15	100.33±1.17	22.33±1.20
Gent 0000 hrs	139.42±1.02	4.30±0.08	101.14±0.70	21.71±0.80

Data is presented as mean ± SEM (n=6-7) and analyzed using One Way ANOVA. No significant differences were observed following One way ANOVA.

Table 2: Effect of chronomodulated gentamicin-artesunate administration on hepatic biomarkers in wistar rats

Group	**ALT IU/l**	**AST IU/l**	**ALP IU/l**
Normal Saline 1200 hrs	71.28±6.72	65.14±7.12	67.00±7.00
Gent 1200 hrs	74.71±7.40	65.28±7.68	130.57±13.40**
Art 1200 hrs	73.85±4.07	62.42±4.20	72.57±9.48
Art+Gent 1200 hrs	69.85±5.02	60.85±5.76	82.28±13.42
Gent+Art 0000 hrs	70.66±8.56	63.16±7.03	76.33±10.01
Gent 0000 hrs	81.14±6.52	70.71±5.37	85.85±6.34

Data is presented as mean ± SEM (n=6-7) and analyzed using One Way ANOVA followed by Hochberg post hoc test. ** = p<0.01 compared with saline.

Table 3: Effect of chronomodulated gentamicin-artesunate administration on serum markers of oxidative markers in wistar rats

Group	**MDA[µmol/l]**	**SOD[U/l]**	**CAT[nmol/l]**	**GPX[U/l]**
Normal Saline 1200 hrs	1.07±0.09	2.15±0.11	53.28±1.04	44.57±1.77
Gent 1200 hrs	1.12±0.06	2.00±0.06	53.00±1.04	47.28±1.61
Art 1200 hrs	1.00±0.05	2.27±0.09	48.57±2.10	51.00±1.97
Art+Gent 1200 hrs	2.07±0.82	2.08±0.10	50.57±1.04	48.14±2.89
Gent+Art 0000 hrs	0.93±0.12	2.31±0.11	54.50±1.72	47.00±.93
Gent 0000 hrs	1.11±0.15	1.95±0.10	50.85±2.14	45.28±1.16

Data is presented as mean ± SEM (n=6-7). No statistically significant difference in means following one way ANOVA.

Table 4: Effect of chronomodulated gentamicin-artesunate administration on serum protein and glucose levels in wistar rats

Group	**TP[g/dl]**	**ALB[g/l]**	**GLUCOSE[mmol/l]**
Saline 1200 hrs	64.71±1.08	35.42±1.13	4.51±0.11
Gent 1200 hrs	66.42±1.92	37.14±1.80	4.75±0.26
Art 1200 hrs	66.71±1.40	39.00±1.57	4.75±0.26
Art+Gent 1200 hrs	66.14±0.59	36.71±0.68	4.74±0.09
Gent+Art 0000 hrs	66.50±1.05	36.33±1.30	4.43±0.16
Gent 0000 hrs	63.14±1.12	35.57±1.02	4.78±0.26

Data is presented as mean ± SEM (n=6-7). No statistically significantdifference in means following one way ANOVA.

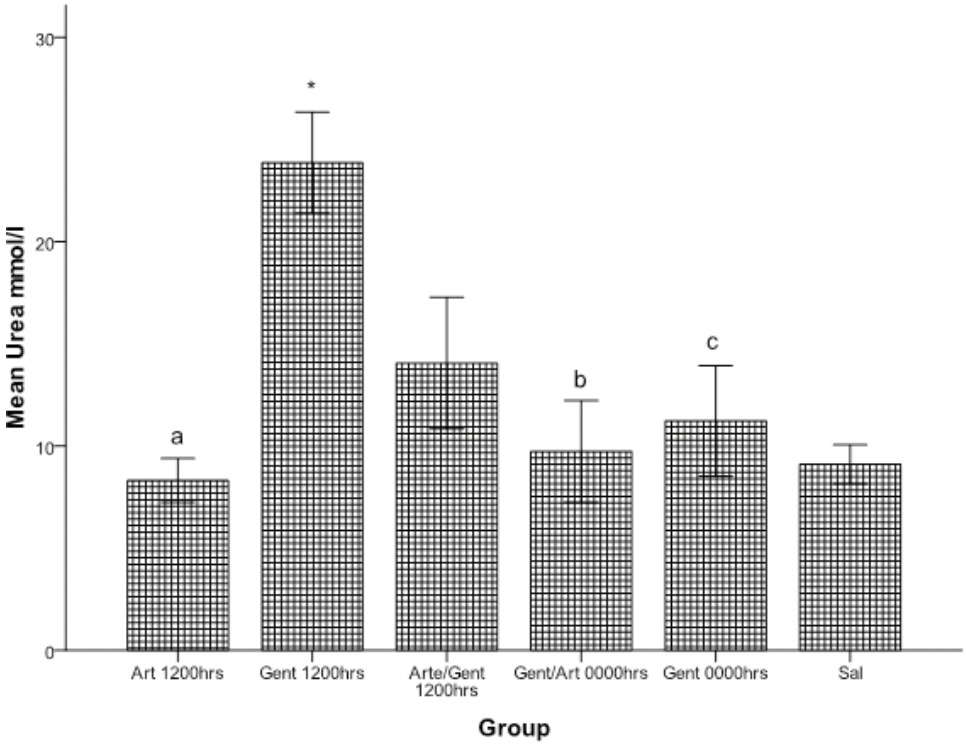

Figure 1: Effect of chronomodulated gentamicin-artesunate administration on serum urea levels in wistar rats.

Data is presented as mean ± SEM (n=6-7) and analyzed using One Way ANOVA followed by Hochberg post hoc test. *: p = 0.001 in comparison with saline control, a, b, c are p values of 001, .003, .008 for in comparison with gentamicin 1200 hrs

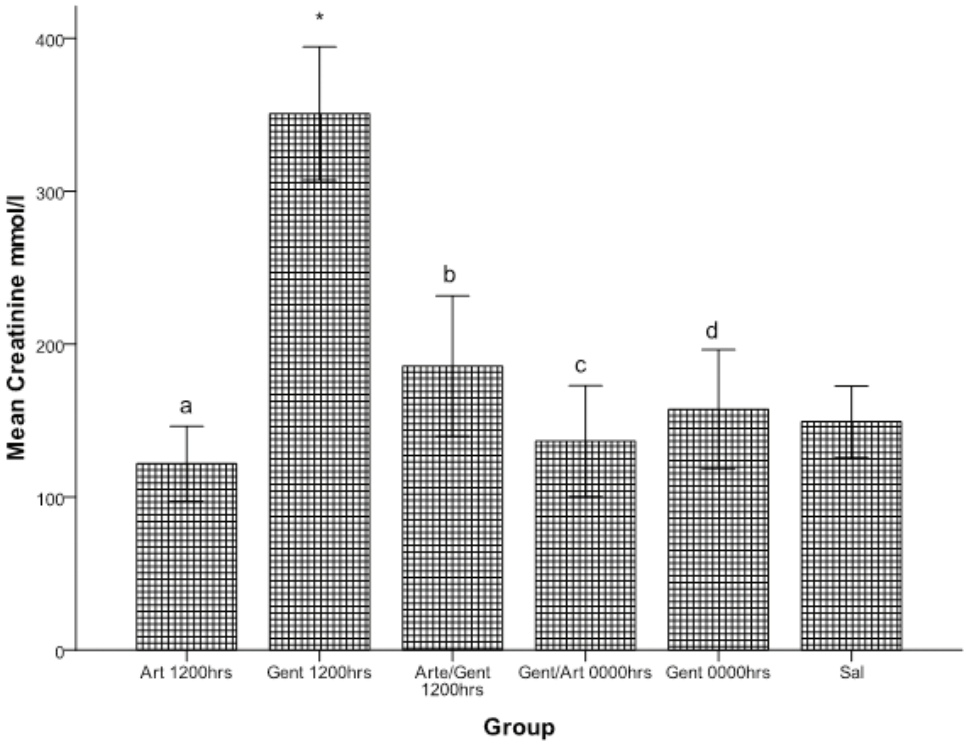

Figure 2: Effect of chronomodulated gentamicin-artesunate administration on serum creatinine levels in wistar rats.

Data is presented as mean ± SEM (n=6-7) and analyzed using One Way ANOVA followed by Hochberg post hoc test.

#=significant difference in comparison to saline; *p=0.007 in comparison with saline control; a, b, c are p values of 0.001, 0.045, 0,005, 0.01 in comparison with gentamicin 1200 hrs

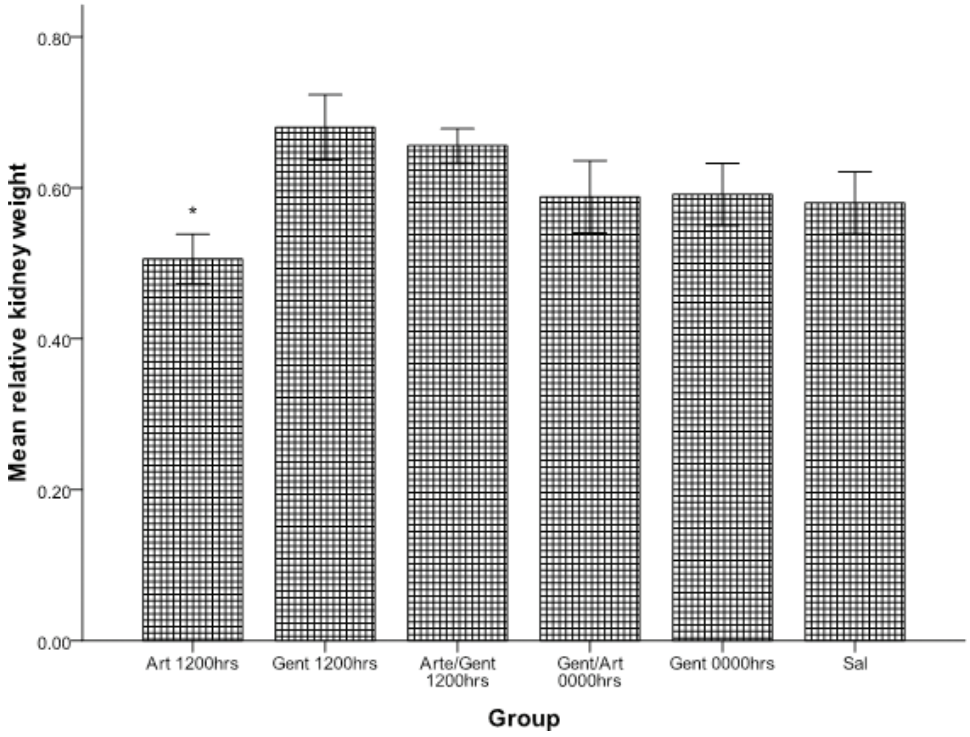

Figure 3: Effect of chronomodulated gentamicin-artesunate administration on relative kidney weight in wistar rats.

Data is presented as mean ± SEM (n=6-7) and analyzed using One Way ANOVA followed by Hochberg post hoc test.
*p=0.05 compared to Art/Gent 1200 hrs

weight ratio when compared with the gentamicin 1200 hrs group (Figure 3).

DISCUSSION

The current study corroborates previously documented reports[21] on the temporal variation in gentamcin toxicity. This was evidenced by the high levels of renal biomarkers at the rest period of the rats (1200 hrs) and low levels at the high activity period (0000 hrs). However, the concurrent administration of gentamicin and artesunate at both periods did not result in any observable deleterious effect on the kidney as was also the case with the liver. Temporal variation in gentamicin nephrotoxicity has been previously demonstrated in rats, and the administration of gentamicin during activity period (being 0000 hrs in this study) has been shown to pose a lower risk of nephrotoxicity due to increased glomerular filtration during this period.[22] Artesunate has been shown to exhibit some adverse effects on the kidney following a 21-day high dose regimen in mice,[23] and also exhibits reversible renal toxicity at a very high intravenous dose.[24] However the concurrent administration of artesunate and gentamicin in this study resulted in amelioration of gentamicin associated nephrotoxicity. Although gentamicin nephrotoxicity is most often oligouric, several diuretics have been reported to ameliorate the nephrotoxicity of gentamicin. A reduction in the accumulation of gentamicin in the kidney is a major approach to decreasing its nephrotoxicity,[25] and the use of single dose and timed administration reduces the quantity and period of contact of the kidney with gentamicin thus reducing nephrotoxicity. Artesunate's diuretic properties have been previously reported.[8,26] This diuretic effect may have contributed to the clearance of gentamicin and reduced the nephrotoxicity at both 0000 hrs and 1200 hrs. Artesunate, in addition to the diuretic effect had also been shown to increase renal blood flow. This may thus reduce the contact period of the antibiotic with the nephrons thus reducing the toxicity. The diuretic frusemide has been previously reported to reduce gentamicin induced nephrotoxicity.[27] Earlier studies also reported that frusemide did not increase the risk of aminoglycoside renal and auditory toxicity.[28,29] The diuretic effect reported by previous researchers may be a possible mechanism behind the amelioration of the gentamicin induced nephrotoxicity. The night time (0000 hrs) administration of gentamicin results in reduced nephrotoxicity. This period is the activity period in rodents and there is a higher level of glomerular filtration in contrast to the rest period. A reduced clearance during rest periods in man and also possibility that the toxicity of gentami-

cin was more likely due to the duration of contact in the kidney rather than the plasma concentrations has been postulated.[30] However, the diuretic effect of artesunate may have equally contributed to the reduction in nephrotoxicity with the 0000 hrs in a similar fashion to that of the 1200 hrs dose which typically exhibits the least toxicity. Thus the available concentration to exert toxicity due to contact is diminished. The kidney sometimes fails acutely as a consequence of malaria or falciparum species infection,[31] and mortality may be as high as 45% when it occurs.[32] Thus the ameliorative effect of artesunate in the face of concurrent malaria and gentamicin sensitive bacteria infection could be advantageous in malaria related kidney failure on artesunate therapy.

CONCLUSION

The nephrotoxicity associated with gentamicin administration was ameliorated in the presence of artesunate both during the active (0000 hrs) and rest (1200 hrs) periods. This could be beneficial in concurrent treatment of bacteria and falciparum co morbid states. Although the exact mechanism by which this occurs remains to be fully elucidated, the diuretic effect of artesunate may be a contributory factor.

ACKNOWLEDGMENT

The authors acknowledge the assistance of Mr. Olu Ayegbusi of the Department of Chemical Pathology, Ahmadu Bello Teaching Hospital, Zaria-Nigeria.

CONFILICT OF INTEREST

The authors declare no conflict of interest.

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