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Impact of Fractionated Gentamicin Dosing on Induction of Nephrotoxicity: An Experimental Study in Rats

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Abstract

Gentamicin (GIN) is a widely used antibiotic, but nephrotoxicity restricts its therapeutic use. GIN affects mainly renal tubules due to presence of megalin/cubulin complex, which is responsible for its uptake in proximal tubular epithelial cells. In this study, we investigated whether administration of GIN dose (100 & 120 mg/kg) once/day or fractionation of everyday dose over 4 hours could produce more nephrotoxic effect in rats. Kidney function was evaluated through assessment of serum and urinarycreatinine (Cr), blood urea nitrogen (BUN), and glomerular filtration rate (GFR).Furthermore, these biochemical estimations were supported by the histopathological study. Administration of GIN 100 & 120 mg/kg once/day for 1 week produced non-significant changes in kidney function biomarkers, in addition to preserved histological structure of glomeruli. However, fractionation of GIN dose 120 mg/kg over 4 hours everydayfor 1 week produced significant deterioration of kidney function, as well as histopathological alterations. Fractionated dosing of GIN every day is more nephrotoxic than administration of the dose once/day. That could be attributed to saturation of megalin/cubulin complexand thus impaired endocytosis of GIN in proximal tubular epithelial cells when administered once daily. Thus, we suggest that administration of GIN in fractionated doses is more suitable for induction of nephrotoxicity in rats.

Keywords: Gentamicin, Fractionated dosing, Megalin/cubulin complex, Nephrotoxicity

1. Introduction

Gentamicin (GIN) antibiotic was first discovered in 1963 that has potent bactericidal activity against Gram-negative bacteria. GIN inhibits bacterial protein synthesis by binding to the 30S ribosomal subunit. However, its therapeutic utility is restricted due to the risk of major side effects, the most prevalent of which are ototoxicity and nephrotoxicity[1].

Tubular cytotoxicity is a key feature of GIN nephrotoxicity. Apoptosis and necrosis of tubular epithelial cells have been observed in experimental animals and culture cells treated with GIN [2]. The elevated expression of transport molecules for proteins and cations (megalin/cubilin complex) in proximal tubules is associated with increased GIN uptake and accumulation in renal cells. This complex participates a crucial role in the transport of GIN through endocytosis [2].

Megalin, also known as gp330 or low-density lipoprotein receptor-related protein, is a member of the low-density lipoprotein receptor family of proteins (LRP2). Megalin is mostly expressed on the apical surface and in apical endocytic compartments of epithelial cells in the S1 segment of the proximal tubule (PT) of the kidney, with lesser amounts in the S2 and S3 segments. Megalin and cubilin are multiligand receptors that coordinate the absorption of most filtered proteins as well as a variety of other small compounds from the glomerular ultrafiltrate[3].

Previous studies have demonstrated the saturation kinetics of GIN uptake into renal cortex, where sustained infusion of GIN resulted in markedly elevated GIN concentration in the renal cortex compared to single dose administration.Saturation of uptake occurs when drug concentrations in the

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tubular lumen at the site of absorption exceed the Km of transport (15 mg/L) [4, 5].

Therefore, this study aimed to investigate whether administration of GIN dose (100 & 120 mg/kg) once/day or fractionation of the dose over 4 hours could induce nephrotoxicity in rats. Kidney function was estimated through assessment of serum and urinary creatinine (Cr), blood urea nitrogen (BUN), and glomerular filtration rate (GFR). Furthermore, these biochemical estimations were supported by the histopathological study.

2. Materials and methods

Animals

Male Wistar rats weighing 180-200 g were obtained from the Nahda Animal Facility at Nahda University in Beni-Suef, Egypt. Rats were housed under regulated settings of room temperature (23 ± 2°C) and 12/12 hours dark-light cycles for two weeks to allow their acclimatization. The rats had unrestricted access to standard diet as well as water. Animal procedures followed the National Institutes of Health guide for care and use of laboratory animals. The Institutional Animal Care and Use Committee at Beni-Suef University has also given its approval to the experimental methods (IACUC, 019-81).

Drugs and kits

In this experiment Garamycin® ampoules (Schering-Plough) containing 80 mg/2 ml GIN sulphate were used. Cr and BUN kits were purchased from Bio-Med (Egypt).

Experimental design

Thirty male Wistar rats weighting 180-200 g were utilized in the study. Rats were divided into five groups, each of six rats. In the first group (control), rats were administrated saline only i.p. In the second and third groups, rats were administrated GIN 100 mg/kg and 120 mg/kg, respectively, once/day for 7 days. In the fourth and fifth groups, rats were administered 25 mg/kg/hr for 4 hours (totally 100 mg/kg/day) and 30 mg/kg/hr for 4 hours (totally 120 mg/kg/day), respectively,i.p. for 7 days. GIN was administered at the same time every day. After 24 hours of the last dose, urine, blood, and kidney samples were collected for biochemical estimation of Cr, BUN, and histopathological analysis.

Collection of urine, serum, and kidney samples

Each rat was placed in a metabolic cage for 24 hours after the last dose of GIN to collect urine. The urine samples were centrifuged at 1000 rpm for 10 minutes before storage at -20ºC to determine Cr.

Blood and kidney tissue samples were taken on the 8th day. Blood samples were centrifuged at 4000 rpm to separate the serum, which was subsequently used to determine Cr and BUN level. Kidney samples were fixed in 10% formol-saline to be used for histopathological examination.

Evaluation of kidney functions

For assessing acute kidney damage, the levels of Cr and BUN in serum and urine samples were estimated by Bio-Medkits according to manufacturer's instructions [6, 7]. The GFR was calculated using the previously reported formula: (Cr in urine × urine volume for 24hr)/Cr in serum×1440 [8].

Histological investigation

Fix kidney samples in 10% formol-saline were subjected to dehydration using serial dilutions of alcohol. That was followed by fixation of specimens in paraffin wax for 24 hours at 56°C in hot air oven. Paraffin blocks (5 µm) were transversely sectioned using sledge microtome, then sections were stained with Hematoxylin and Eosin (H&E) [9].

Statistical analysis

All the data were presented as means with standard errors (S.E). GraphPad Prism 8 (GraphPad Software Inc., USA) was used to perform all statistical analyses using one-way analysis of variance (ANOVA) followed by Dunnett’s post-hoc test. At p 0.05, the results were considered statistically significant.

3. Results

The effect of once and fractionated daily dosing of GIN on serum Cr level

There was no significant change in Cr level between the control, GIN (100 mg/kg), GIN (120 mg/kg) once/day dosing, and GIN (100 mg/kg) fractionated dosing groups. However, there was a 4.42-fold rise in serum Cr level between the control and GIN (120 mg/kg) fractionated dosing group (Fig 1).

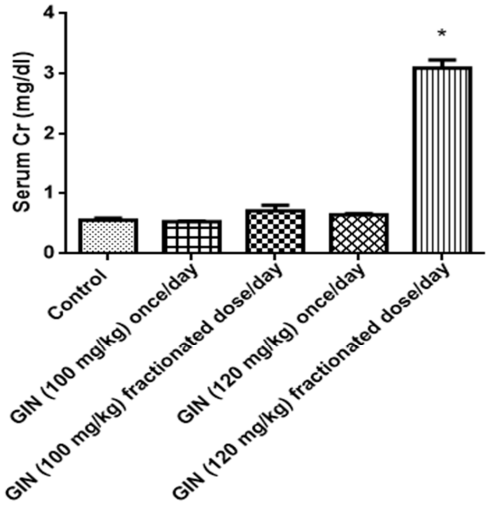

Fig. 1: Effect of once and fractionated daily dosing of GIN on serum Cr level. Fractionated dosing of GIN 120 mg/kg significantly elevated serum Cr level compared to the control, while once daily dosing produced non-significant change. The data are expressed as mean ± S.E. (n=6). Comparisons were performed using one-way ANOVA followed by Dunnett's post hoc test.* Significantly different from the control group at $p < 0.05$.Cr, creatinine; GIN, gentamicin

The effect of once and fractionated daily dosing of GIN on urinary Cr level

Rats administrated GIN (100 mg/kg) once/day, GIN (100 mg/kg) fractionated dosing, GIN (120 mg/kg) once/day dosing groups didn't show a significant change in urinary Cr level. However, in GIN (120 mg/kg) fractionated dose/day group, there was 48.55% decrease in urinary Cr level when compared to the control group (Fig 2).

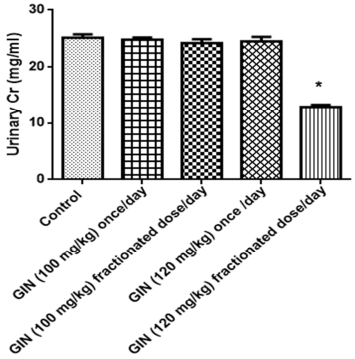

Fig. 2: Effect of once and fractionated daily dosing of GIN on urinary Cr level. Fractionated dosing of GIN 120 mg/kg significantly elevated urinary Cr level compared to control, while once daily dosing produced non-significant change. The data are expressed as mean ± S.E. (n=6). Comparisons were performed using one-way ANOVA followed by Dunnett's post hoc test.* Significantly different from control group at $p < 0.05$.Cr, creatinine; GIN, gentamicin

The effect of once and fractionated daily dosing of GIN on BUN level

Our results showed that in rats administrated GIN (100 mg/kg) once/day, GIN (100 mg/kg) fractionated dose/day, and GIN (120 mg/kg) once/day BUN level was significantly increased by 1.22-fold, 1.19-fold, and 1.64-fold, respectively. But in GIN (120 mg/kg) fractionated dose/day group, BUN was markedly increased by 2.71-fold when compared to the control group (Fig 3).

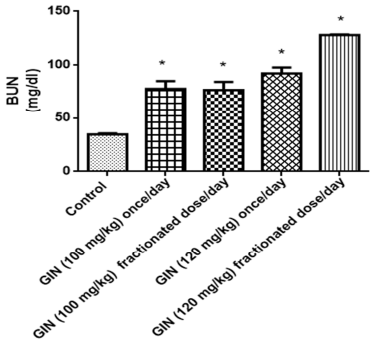

Fig. 3: Effect of once and fractionated daily dosing of GIN on BUN level. Once and fractionated daily dosing of GIN 100 & 120 mg/kg significantly elevated BUN level compared to the control. The data are expressed as mean ± S.E. (n=6). Comparisons were performed using one-way ANOVA followed by Dunnett's post hoc test.* Significantly different from the control group at $p < 0.05$.BUN, Blood urea nitrogen; GIN, gentamicin.

The effect of once and fractionated daily dosing of GIN on GFR

Regarding GFR, GIN (120 mg/kg) fractionated dose/day group showed a significant reduction in GFR by 88.03% when compared to the control group, while no significant change was observed between GIN (100 mg/kg) once/day group, GIN (100 mg/kg) fractionated dose/day group, or GIN (120 mg/kg) once/day group and the control group (Fig 4).

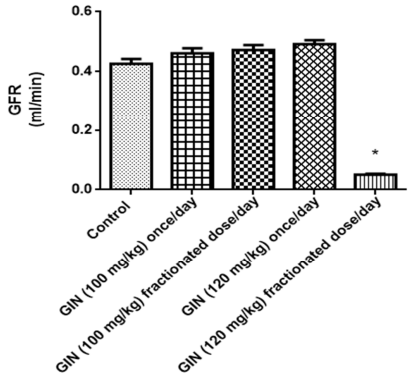

Fig. 4: Effect of once and fractionated daily dosing of GIN on GFR. Fractionated dosing of GIN 120 mg/kg significantly decreased GFR compared to the control, while once daily dosing produced non-significant change. The data are expressed as mean ± S.E. (n=6). Comparisons were performed using one-way NOVA followed by Dunnett's post hoc test.* Significantly different from the control group at $p < 0.05$.GFR, glomerular filtration rate; GIN, gentamicin

Effect of GIN on histopathological changes

Normal kidney architecture was observed in normal control group (Fig 5A). Sections that were obtained from GIN (100 and 120 mg/kg/day) once/day groups showed normal glomeruli and bowman's capsules but scattered necrotic proximal tubules (Fig 5 B & C). However, in fractionated dosing GIN (120 mg/kg/day) group, the kidney showed an irregular renal capsule, distorted hypercellular glomeruli with obliterated bowman's spaces, marked tubular necrosis, proximal tubules with apoptotic epithelial lining (Fig 5D). The histopathological scoring is presented in Table 1.

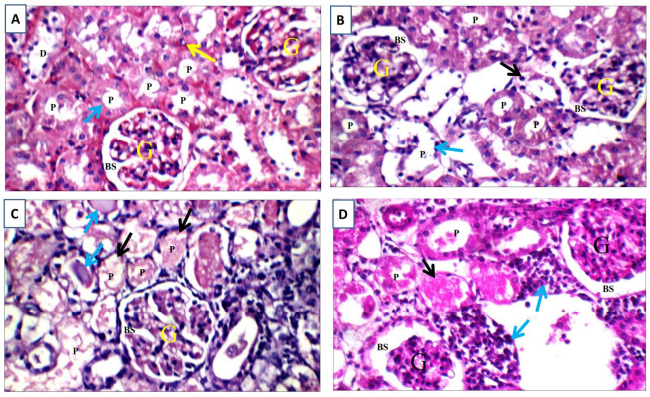

Fig. 5: Images of kidney sections from rats administered once and fractionated daily dosing of GIN (A)kidney showing average glomerulus (G) with average Bowman's space (BS), average proximal tubules (P) with preserved brush borders (black arrow), average distal tubules (D), and average interstitium (yellow arrow) (H&E X 400), B) GIN (100 mg/kg) once daily dosing group showing average glomeruli (G) with average Bowman's space (BS), scattered proximal tubules (P) with edematous epithelial lining (black arrow) and complete loss of brush borders (blue arrow) (H&E X 400), C) GIN (120 mg/kg) once daily dosing group showing average glomerulus (G) with average Bowman's space (BS), scattered completely necrotic proximal tubules (black arrows) with intra-tubular hyaline casts (blue arrow) (H&E X 400), D) GIN (120 mg/kg) fractionated daily dosing group showing hypercellular glomerulus (G) with wide Bowman's space (BS), marked tubular necrosis (black arrow), and marked interstitial inflammatory infiltrate (blue arrow) (H&E X 400).

Table 1

Histopathological scoring

Groups	Glomeruli	Boman's spaces	Tubules		
			lining	Brushborder	lumen
Normal control	0	0	0	0	0
GIN (100 mg/kg) once dose/day	0	0	+	++	0
GIN (120 mg/kg) once dose/day	0	0	+	++	0
GIN (100 mg/kg)fractionated dose/day	0	0	+	++	0
GIN (120 mg/kg)fractionated dose/day	++	+	++	++	++

Glomeruli: 0: Normal +: Edematous/congested ++: Small-sized/atrophied

Bowman's spaces (BS):0: Normal +: Widened/dilated ++: Narrow/obliterated

Tubules:

- Lining: 0: Normal+: Mild apoptosis ++: Moderate/marked necrosis
- Brush border: 0: Preserved +: Partial loss ++: Complete loss
- Lumen: 0: Free +: Intra-tubular debris ++: Intra-tubular casts

4. Discussion

Due to the increased perfusion to kidney and excretion of toxic compounds, kidneys are more susceptible to damage especially in tubular cells [10].

Gentamicin antibiotic still has a crucial role in treatment of life-threatening infections. But GIN's clinical usage is limited due to its nephrotoxicity [11, 12]. Over 90% of GIN dosage is excreted by the kidneys, with urine concentrations ranging from 50 to 200 g/mL [13].

Elevated levels of BUN and Cr in serum and urine levels are considered as the main criteria for the diagnosis of acute kidney injury (AKI) or nephrotoxicity [14].Cr is a small molecule that is constantly created by the body as a waste product from muscles, filtered by the glomeruli, and not reabsorbed or secreted by the tubules. In AKI, serum Cr rises due to reduced glomerular filtration and back leak via injured proximal tubular cells. After kidney damage, the Cr level rises gradually, typically matching the AKI criteria [15].

In addition, GFR is considered the most extensively used measure of kidney function in animals with kidney disease across the world. GFR is an approximate estimation of the number of functional nephrons by estimating their rates of filtration. Creatinine clearance (CrCl) is an index of GFR and renal blood flow. Serum Cr and timed urine collection (for example, a 24-hour urine collection) can be used to calculate CrCl[16, 17].

The findings of our investigation revealed that i.p. treatment with 100 mg/kg and 120 mg/kg GIN once daily for 7 days caused tubular necrosis but had no effect on the glomerular system. This was confirmed by histological investigation, which revealed normal glomeruli as well as serum Cr level that did not alter considerably following GIN administration. On the other hand, we found that fractionated dose of GIN (120 mg/kg) over 4 hours caused considerable decrease in kidney function, that was also confirmed by histopathological findings.

The tubular necrosis observed after once daily dosing of 100 and 120 mg/kg of GIN affects mainly proximal tubules, that could be attributed to presence of megalin/cubulin complex [18, 19, and 20]. Regarding glomerular system, it has been previously demonstrated that glomerular toxicity occurs after exposure to large doses of GIN [2, 21]. Despite 100 and 120 mg/kg are large doses, glomerular damage wasn't observed. This could be explained depending on GIN kinetics,where GIN uptake by megalin/cubulin complex has been previously shown to be prone to early saturation in inner ear and renal cortex. This observation has been confirmed by comparing GIN concentration after single i.m. injection of 10 or 100 mg/kg and 3 h constant infusion of 15 µg/min, where there was failure to detect stable concentration of GIN after single i.m. injection when compared to infusion [4]. Also, Giuliano, Verpooten et al. [5] have attributed the difference between GIN concentration in renal cortex and the stable serum concentration after 6 hours continuous infusion to uptake saturation. Consequently, it has been revealed previously that administration of GIN at a single high dose reduces its nephrotoxic effect compared to continuous infusion [22].

Our experiment emphasized that fractionation of GIN dose is more effective than once daily dosing for induction of nephrotoxicity in rats as demonstrated by marked deterioration of kidney function biomarkers as well as histological structure.

5. Conclusion

Fractionated dosing of GIN everyday induces nephrotoxicity in rats more effectively than once daily dosing. That could be attributed to saturation of megalin/cubilin complex, whichis responsible for the uptake and accumulation of GIN, leading to impaired endocytosis of GIN in proximal tubular epithelial cells. GIN 120 mg/kg fractionated daily dosing for 7 days is more suitable for induction of nephrotoxicity in rats compared to single daily dosing.That could be evidenced by the deterioration of kidney function biomarkers (BUN, Cr, GFR) as well as the marked histopathological alterations at both the glomerular and tubular levels.

6. Conflict of interest

The authors declare that there is no conflict of interest in this review.

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