Effect of gentamicin on the histology of renal tubules in different doses, Experimental study

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الخلاصة

بينت الدراسة الحالية ان الجنتامايسين يسبب تأثير طفيف على النبيبات الكلوية القريبة بجرعة 5 ملغم \كغم لمدة 14 يوم، تمثلت هذه التغيرات بفقدان للحافة الفرشاتية . ظهور فجوات مختلفة الاحجام مع تسطح و توسع النبيبات، كما لوحظ تكثف الكروماتينات النووية مع انفصال جزئي للخلايا من الغشاء القاعدي. بالمقارنة، لوحظ التاثير الكبير الواضح للجنتامايسين بجرعة 10 ملغم\كغم والتي اظهرت فقدان للسطح القمي وانسلاخ الخلايا من الغشاء القاعدي، وكذلك الفقدان شبه الكامل للحافة الفرشاتية وزيادة في عدد وحجم الفجوات السايتوبلازمية. الكثير من النوى ذات الكروماتين المكثف والخلايا ذات النوى المتشددة لوحظت تتموضع في تجويف النبيبات.
ان للجنتامايسين تأثير سلبي على انسجة الكلية والنبيبات الكلوية خصوصا في الجرعات العالية ، مقارنة بالجرعات المنخفضة التي اظهرت اضرارا طفيفة.

Abstract

The present study showed that the gentamicin cause a mild effect to the proximal convoluted tubules at dose of 5 mg/kg for 14 days represented by loss of brush border. Vacuoles of various sizes appeared with flattening and dilatation of the tubules, condensation of nuclear chromatin was observed, with partial detachment of the cells from the basement membrane. In comparison the more clear effect of gentamicin appears at 10 mg/kg dose that included loss of apical surface and detachment of cells from the basement membrane, with nearly complete loss of brush border and increase in number & size of the cytoplasmic vacuoles. Many nuclei with condensed chromatin and nuclear fragmented cells (apoptosis) were located in tubular lumen.

The study concluded that gentamicin has adverse effect on the kidney and renal tubules histology especially in the high dose administration, comparison with low dose that reveal to mild damage.

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Introduction

Aminoglycoside antibiotics are often used for the treatment of severe infections due to Gram-negative bacteria, but they may cause nephrotoxic reactions [1, 2]. The aminoglycosides (Gentamicin, neomycin, Streptomycin) and fluoroquinolones (Ofloxacin) are routinely used by fertility specialists to treat bacterial infections occurring prior to *in vitro* fertilization treatment, or when high concentration of leukocytes are present in the semen of these patients, irrespective of microbial evidence of infection[3] .

Recent studies using rats treated with therapeutic doses of Gentamicin confirmed the observations in humans regarding the adverse effects of aminoglycosides on spermatogenesis [4].

The present study designed to explain the effect of gentamicin in two dose levels intraperitoneally on the kidney especially the proximal convoluted tubules (PCT).

Materials and methods

A total of 15 adult <u>Rattus</u> <u>norvegicus</u> <u>albinus</u> male rats were 8 weeks old and weight 250 ± 10 g., that were obtained from animal house of embryological research and infertility treatment institute of Al-Nahrain University.
Thereafter the rats were randomized into control (n= 5) and experimental (n= 10) groups.

The experimental groups were subdivided into two groups; one of them received 10 mg/kg and the other one received 5 mg/ kg, the two doses of gentamicin administered intraperitoneally daily for fourteen days, respectively.

The animals were administered pentobarbital sodium (40 mg/kg) intra peritoneally for anesthesia. A midline abdominal incision was made, and the kidneys removed and fixed with 10% formalin fixative for 24 hrs then dehydrated with ascending concentrations of alcohol and clearing with xylene, embedding in the paraffin wax to form the tissue blocks, the sectioning of the tissue was done at 4 μm, and the sections stained with

Heamatoxylin and eosin (H&E)[5]and periodic acid – Schiff method
(PAS)[6].

Results

In control group the results revealed that the PCT is lined by
simple cuboidal epithelial cells with round or oval centrally located
nuclei. Prominent brush border of microvilli projected into and
occluded the lumen (figure 1), the brush border and basement
membrane were given strongly PAS – positive (figure 2).

**Figure (1): section in corticomedullary junction of rat kidney in control
group showing the normal tubule, with normal nuclei (n), occluded lumen
(L)H & E, (X1000) 2.8**
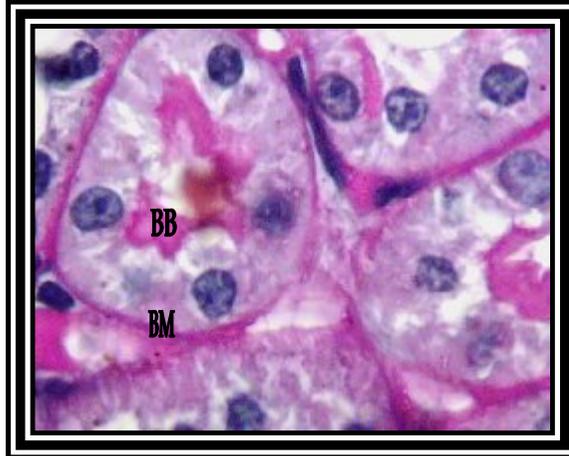
Figure (2): section in cortecimedullary junction of rat kidney in control group showing normal basement membrane (BM), normal brush border (BB)PAS, (X1000) 2.8

In comparison to kidney prepared for light microscopic examination and stained with H & E and PAS in the group of rats that administered 5 mg/kg gentamicin were examined. Structural alterations in PCT cells include loss of brush border. Vacuoles of various sizes appeared with flattening and dilatation of the tubules, condensation of nuclear chromatin was observed, with partial detachment of the cells from the basement membrane in sections stained with H&E and PAS, respectively (figure 3& 4).
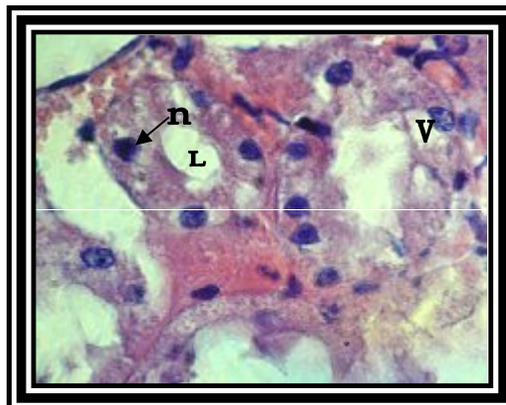
Figure (3): section in corticomedullary junction of rat kidney in administered 5 mg/kg gentamicin group showing large lumen (L), vacuoles (v), condensed nuclei H&E, (X 1000) 2.8

Figure (4): section in corticomedullary junction of rat in administered 5 mg/kg gentamicin group showing large lumen (L), basement membrane (BM) ,brush border (BB) PAS ,(X1000) 2.8

The dearrangement of tubular cells was clear and more tissue damage seen in the group of rats administered 10 mg/kg gentamicin. There were further loss of apical surface and detachment of cells from the basement membrane, with nearly complete loss of brush border and an increase in number & size of the cytoplasmic vacuoles. Many nuclei with condensed chromatin and nuclear fragmented cells (apoptosis) were located in tubular lumen in section stained with H&E, and PAS, respectively (figure 5 & 6).
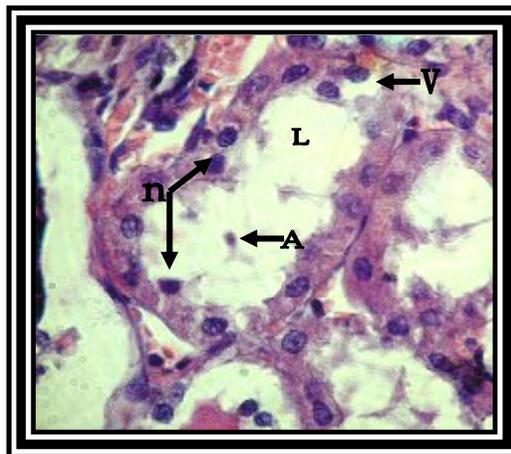
Figure (5): section in corticomedullary junction of rat kidney administered 10 mg/kg gentamicin showing large lumen (L) ,vacuoles (V) ,condensed nuclei (n) ,apoptotic body (A) H&E (X 1000) 2.8
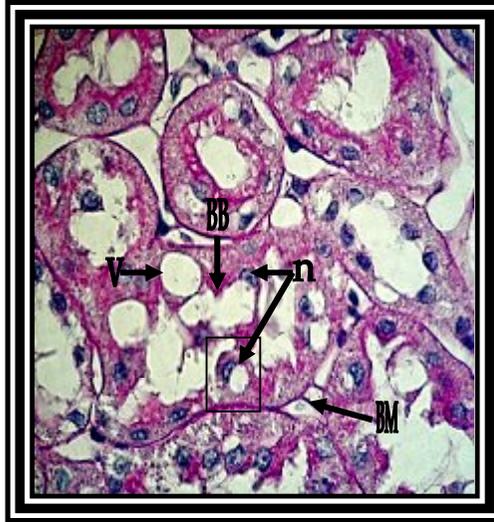
Figure (6) :section in corticomedullary junction of rat kidney administered 10 mg/kg gentamicin showing large lumen (L) ,basement membrane (BM) , brush border (BB) ,vacuole(v) , cresent shape nuclei (n) PAS (X1000) 2.8

Discussion

The present study unambiguously showed that gentamicin caused defect and alteration in the general histological structure of the proximal convoluted tubule as compared with control group, the renal damage that referred in the results of the present work according to the dose of administered gentamicin were graduated from mild changes to severe damage in addition to apoptosis and destruction of cells (illustrated in fig. 3, 4, 5, and 6, respectively)

These results interpreted by the previous findings, that showed gentamicin causes expansion of interstitial space and intertubular space with vacuolization, degeneration, fibrosis and necrosis of interstitial (Leydig) cells following exudation into the interstices was developed and congestion in veins [7] , also gentamicin showed that mitochondria lost cristae and lyzosomes were seen clearly in cytoplasm of sertoli cells, nuclei of myoied cell with heterochromatin which has confirmed stage of cell death [8] .

Apoptosis is thought to develop in 2 distinct phases, occurring in succession; namely a first, reversible phase of commitment, which is under the control of death antagonists and agonists like the members of the Bcl-2 protein family, and is associated with the activation of a certain number of cysteine-aspartate - specific proteases (caspases), mainly caspases 2, 8, 9, and 10, and a second, irreversible phase of execution, which involves the activation of other caspases, mainly caspases 3, 6, and 7. [9]

The other studies explain the animals treated at large with supra-therapeutic doses of gentamicin show extensive necroses of proximal tubules [10] , those receiving low, more clinically relevant doses show a marked proliferation and de-differentiation of renal proximal tubules without evidence of necrosis [11,12].

Cell fractionation and morphometric studies have shown that gentamicin pinocytosed by fibroblasts and accumulated in lysosomes reaches, therein, concentrations at least 30-fold larger than in the extracellular medium [13], where part of the drug may traffic to the Golgi vesicles [14], but animal studies have revealed a huge accumulation of gentamicin in lysosomes of proximal tubular cells [15], suggesting that this property is probably in general. Gentamicin stored in lysosomes, as well as in Golgi vesicles, is expected to become fully protonated and therefore strongly polycationic, due to the acidic pH (5 to 6) prevailing in these compartments [16].

One of the most early and conspicious alterations related to the accumulation of gentamicin in lysosomes was the development of a phospholipidosis which is directly related to the capacity of the intralysosomal polycationic drug to bind to phospholipid bilayers [17]. It may be envisioned that the large concentrations of gentamicin in lysosomes will eventually cause a destabilization of their membrane resulting in their *in situ* disruption, which, by itself, is likely to trigger apoptosis.

The lysosomal destabilization triggers mitochondrial events such as the so-called "permeability transition" [18] and/or the release

of the apoptosis -inducing-factor (AIF), which will then cause apoptosis in a Bcl-2-preventable or retard able manner [19].

Finally, these findings draw the conclusion of the adverse effect of gentamicin on the renal tubules especially the proximal convoluted tubules with increase the damage until appears of apoptosis in the renal cells with increase the dose given of gentamicin.

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