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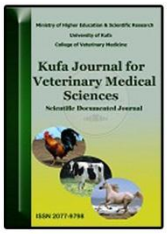

Assessment of Nephroprotective role of Irbesartan against gentamicin induced nephrotoxicity in rats

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Abstract:

Aims of study : The present study was undertaken to assess the renoprotective effect of irbesartan on gentamicin induced nephrotoxicity in male rats.

Materials and methods: Fifteen male adult Sprague-Dawely rats were enrolled in this study, rats were separated randomly into 3 groups, five rats in each group, the first group maintained on normal standard chow diet, served as control group. The second group received gentamicin 100 mg/kg/day, i.p for 4 weeks. The third group received gentamicin 100mg/kg/day i.p concomitantly with irbesartan 25 mg/kg/day p.o for 4 weeks**.**

Results: Gentamicin treatment increased serum urea, creatinine and tissue malondialdehyde (MDA) significantly. Irbesartan treatment decreased serum urea, creatinine and tissue MDA significantly.

Conclusion: Gentamicin induced nenephrotoxicity can be prevented by coadministration with irbesartan.

Key wards: gentamicin, irbesartan , serum urea, creatinine, MDA, renal histopathology.

تقييم دور الإربيزارتان في حماية الكلى ضد التأثير السمي للجنتامايسين على وظائف الكلى في الجرذان

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الخلاصة:

الأهداف: أجريت هذه الدراسة لتقييم التأثير الإيجابي لعقار الإربيزارتان في حماية الكلى لتقليل التأثير السمي للجنتامايسين على وظائف الكلى في ذكور الجرذان.

منهجية البحث : شملت الدراسة خمسة عشر جرذ ذكر بالغ ،وزعوا عشوائيا الى ثلاث مجاميع، كل مجموعة تتألف من خمسة جرذان، المجموعة الأولى بقيت على الغذاء العادي القياسي واعتبرت كمجموعة سيطرة، المجموعة الثانية

أعطيت جنتامايسين بجرعة قدرها 100 ملغم| كغم| يوم تزرق داخل الغشاء البريتوني للجرذ ولمدة 4 أسابيع. المجموعة الثالثة أعطيت جنتامايسين100 ملغم| كغم| يوم تزرق داخل الغشاء البريتوني بالتزامن مع الإربيزارتان بجرعة قدرها 25ملغم \ كغم \ يوم. بعد فترة العلاج أجريت التحاليل المختبرية وهي قياس اليوريا والكرياتنين في الدم ونسبة المالونديلدهايد ودراسة التحاليل النسيجية للكلى في المجاميع الثلاثة وتمت مقارنة النتائج بين هذه المجاميع بالطرق الإحصائية المعتمدة علميا.

النتائج: الجنتامايسين تسبب في زيادة نسبة اليوريا ، الكرياتنين و المالونديلدهايد بصورة معنوية ،الإربيزارتان قلل نسبة اليوريا ، الكرياتنين و المالونديلدهايد بصورة معنوية.

الاستنتاج: بالإمكان منع التأثير السمي للجنتامايسين على وظائف الكلى بالاستخدام المتزامن للإربيزارتان.

Introduction:

Aminoglycosides including gentamicin are very important agents for the treatment of gram negative bacterial infections(1). Aminoglycosides are bactericidal, accumulated intracellularly in microorganisms via an 02-dependent uptake; thus, anaerobes are innately resistant(2), they work by binding the 30S subunit of the bacterial ribosome, interrupting protein synthesis(1).The major side effect of aminoglycosides is Nephrotoxicity, accounting for 10-15% of all cases of acute renal failure(3),although a clear recognition of the patient- and treatment-related risk factors (4), combined with the once-a-day schedule and effective monitoring procedures (5), have definitely improved the situation , we are still short of having brought the safety of aminoglycosides to that of the main other wide-spectrum antibiotics. Gentamicin, like other aminoglycosides, causes nephrotoxicity by inhibiting protein synthesis in renal cells. This mechanism specifically causes necrosis of cells in the proximal tubule, resulting in acute tubular necrosis which can lead to acute renal failure (6) . Irbesartan a non-peptide antagonist, selectively blocks Type 1 angiotensin-II receptors, without increasing the levels of bradykinin and without decreasing of plasma levels of angiotensin-II and aldosterone. Moreover, since angiotensin-II can be produced in alternative metabolic pathways (7),such molecules can achieve a more complete angiotensin blockade than ACE-inhibitors , therefore irbesartan is indicated for the treatment of hypertension and cardiac failure(8). Irbesartan is also shown to delay progression of diabetic nephropathy which is characterized by the early hypertrophy of both glomerular and tubular elements, thickening of the glomerular and tubular basement membranes(9). So irbesartan is indicated for the reduction of renal disease progression in patients with type 2 diabetes(10),hypertension and microalbuminuria (>30 mg/24 hours) or proteinuria (>900 mg/24 hours)(11).It is found that. Irb exerted a renal protective role independently of its antihypertensive effect(12) ,the protective action of irbesartan might be mediated, at least in part, by its effect on tissue oxidant/antioxidant status(13) and possibly through inhibition of renal hypertrophy(14) . The aim of this study is to assess the nephroprotective role of irbesartan aginst gentamicin toxicity in rats through the examination of renal histopathology, MDA level and measurement of serum uria and creatinine.

Materials and methods:

Fifteen male adult Sprague-Dawely rats were enrolled in this study. The animals were obtained from the Animal House in Kufa Medical College. Their weight range was between 50-100 g and aged between 2.5-3.5 months. The rats were housed in Kufa Medical College Animal and kept at 25 ˚C and 12 hours light-dark cycles with 12.00 AM being the mid dark period. Rats had free access to drinking water and libitum. After 1 week of adaptation the rats were separated randomly into 3 groups, five rats in each group as follow :

Group 1 maintained on normal standard chow diet, served as control from which the baseline value of experimental parameters was determined.

Group 2 received gentamicin 100 mg/kg/day, i.p for 4 weeks(15).

Group 3 received gentamicin 100mg/kg/day i.p concomitantly with Irbesartan 25 mg/kg/day p.o for 4 weeks(16). At the end of the 4 weeks blood samples were taken from all rats which underwent laparotomy, and experimental parameters were measured .

Biochemical assay:

After 4 weeks of treatment, 3 ml of blood was obtained directly from the heart of the anesthetized animal (with chloroform) which underwent laparotomy, the blood was placed in serum tube and left to stand for 30 minutes. The serum was prepared by centrifugation at 3000 xg for 10 minute, serum was obtained for determination of experimental parameters urea and creatinine. Urea was estimated according to procedure supplied by the kit of Biomerieux company (17), creatinine was estimated according to procedure supplied by the kit of Syrbio company(18). In addition to that both kidneys were removed from each rat, one of them was kept in 10% formalin for histopathological study and the other one was freezed in deepfreeze (-80ºC) to be used for the determination of tissue malondialdehyde (MDA) level according to the method of Tomotsu et al(19).

Drugs used in the experiment:

Gentamicin: it was used in a dose of 10 mg/kg/day i.p., ampoule contains gentamicin 80mg/2ml **(Megental [Menarini International; Italy]** was used the dose was given to the rats according to the body weight once daily every day for 4 weeks.

Irbesartan**:** it was used in a dose of 25 mg/kg/day p.o., a tablet contains 75 mg Irbesartan **(Aprovel [Sanofi Aventis; France])**, was dissolved in water and the dose was given to the rats according to the body weight once daily every day through stomach tube for 4 weeks.

Statistical analysis: The data expressed as mean ± SEM unless otherwise stated. Statistical analysis had been done by using independent t-test. Significant difference was set at α=0.05.

Results:

Effect of Gentamicin treatment on the selected parameters: serum urea, creatinine and tissue MDA increased significantly (P<0.05) after 4 weeks of Gentamicin treatment **(table 1).**

Table (1): effect of 4 weeks Gentamicin treatment on serum urea, creatinin and tissue MDA in male rat serum (No.=5 rats in each group).

	Normal control group	**Gentamicin treated group**	**P.value**
Urea mg/dl	34.7±0.58	60.8±2.71	<0.05
Creatinine mg/dl	0.34±0.068	0.78±0.037	<0.05
MDA nmol/mg	0.69±0.028	2.94±0.053	<0.05

The values expressed as Mean±SEM

Effect of Irbesartan against Gentamicin treatment on the selected parameters: serum urea, creatinin and MDA decreased significantly (P<0.05) after 4 weeks Irbesartan + Gentamicin treatment **(table 2).**

Table(2): effect of 4 weeks Irbesartan + Gentamicin treatment on urea, creatinin and MDA in male rat serum (No.=5 rats in each group).

	Gentamicin treated group	**Irbesartan treated group**	**P.value**
Urea mg/dl	60.8±2.71	42.81±2.80	<0.05
Creatinine mg/dl	0.78±0.037	0.30±0.07	<0.05
MDA nmol/mg	2.94±0.053	1.45±0.08	<0.05

The values expressed as Mean±SEM

Effect of Irbesartan against Gentamicin treatment on renal histopathology: significant pathological changes has been observed in gentamicin treated group characterized by the presence of vascular congestion and hemorrhage (figure 2) , as compared with the normal renal tissue(figure 1) , while irbesartan treated group revealed mild vascular congestion with no evidence of hemorrhage (figure 3).

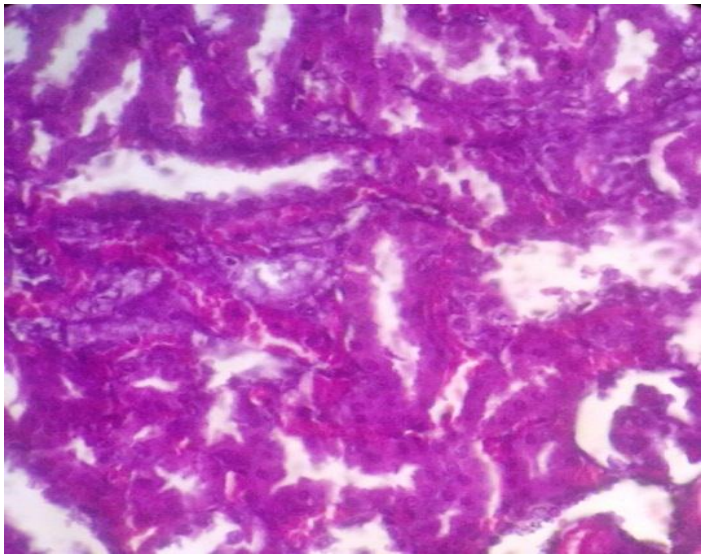

Figure (1) : Normal renal parenchyma

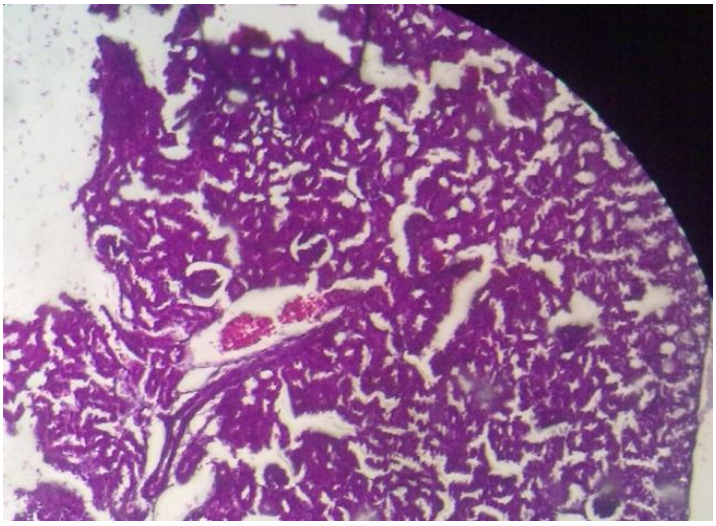

Figure (2) : Gentamicin treated group
Evidence of vascular congestion and hemorrhage

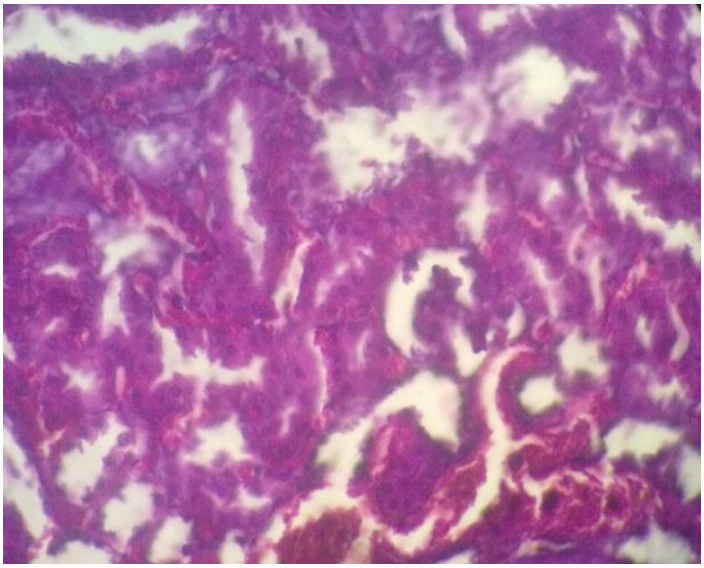

Figure (3) : Irbesartan treated group
Mild vascular congestion with no evidence of hemorrhage

Discussion:

The present study showed that the administration of gentamicin to rats once daily for 30 days reduces glomerular function, as reflected by increased serum creatinine concentrations as well as urea and MDA. Aminoglycoside-induced nephrotoxicity is characterized by a decrease in the glomerular filtration rate(GFR) and direct tubular injury. The interaction between the cationic aminoglycoside and membrane anionic phospholipids is considered to be the first cytotoxic step.

Some studies suggest that aminoglycoside antibiotics can stimulate the formation of ROS(reactive oxygen species), which may be directly involved in gentamicin-induced acute renal failure and membrane lipid peroxidation. It has been found that O2°, hydrogen peroxide (H2O2) and hydroxyl radicals increase with gentamicin-treatment and H2O2 and O2° induce mesangial cells contraction, alter the filtration surface area and modify the ultrafiltration coefficient, factors that decrease the GFR. Therefore, some antioxidants had protective effect on gentamicin induced nephrotoxicity (Ademuyiwa et al., (20) ; Ali(21) .).

Soliman et al(22). and Al-Majed et al.(23) used gentamicin at dose 80 mg/kg for experimental nephrotoxicity in rats and their results were similar to that of our study. Patil et al.(24) applied gentamicin at dose 100 mg/kg for nephrotoxicity in rats and their results were similar to our result. Poormoosavi et al.(25) applied gentamicin at dose 80 mg/kg for nephrotoxicity in rats and their results were similar to our result.

Cuzzocrea et al.(26) investigated the potential role of the superoxide anion in gentamicin-induced renal toxicity by using M40403, low molecular weight synthetic manganese that selectively removes superoxide. They observed a significant increase in kidney myeloperoxidase activity and lipid peroxidation in gentamicin-treated rats. Mazzon et al.(27). demonstrated that N-normalized serum MDA concentrations in gentamicin-induced nephropathy in rats.

Kadkhodaee et al.(28). evaluated the effects of cosupplementation of vitamins E and C on gentamicin induced nephrotoxicity in rats and demonstrated that vitamin C prevented increases in urine lactate dehydrogenase, alkaline

phosphatase and N-acetyl–D-glucosaminidase but did not prevent decrease in renal glutathione concentration and filtration failure. Melatonin prevents the tubular necrosis induced by gentamicin in rats, presumably because it is a potent antioxidant and restores antioxidant enzyme activity in the rat kidney (Ozbek et al.(29)).

Conclusion:

Gentamicin induced nenephrotoxicity can be prevented by coadministration with irbesartan.

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