Journal of Animal and Veterinary Advances 10 (24): 3312-3315, 2011
ISSN: 1680-5593
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Protective Effects of Ampicillin on the Gentamicin-Induced Nephrotoxicity in Rat

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Abstract: Gentamicin is widely used for the treatment of gram negative bacterial infections. Its clinical use however is limited due to its side effects such as nephrotoxicity. Present study aimed to study the protective effect of ampicillin on gentamicin induced nephrotoxicity in rat. For this purpose, 40 male rats were randomly divided in four groups. Group 1 was used as control. In group 2, 40 mg kg^{-1} gentamicin was given intramuscularly for 10 consecutive days. Rats in group 3 received 40 mg kg^{-1} gentamicin and 50 mg kg^{-1} ampicillin intramuscularly. Rats in group 4 were given 50 mg kg^{-1} ampicillin intramuscularly. About 1 day after last injection, rats were weighted, anesthetized and blood samples and histopathological samples of kidney were collected. Marked increases in BUN and serum creatinine levels were observed in gentamicin treated group ($p<0.05$). Co-administration of ampicillin decreased the rise in BUN and serum creatinine level. The most observed abnormality in rats treated with gentamicin was severe acute tubular necrosis and tubular epithelial loss resulting in tubular cast formation. In conclusion these results indicate that co-administration of ampicillin along with gentamicin prevents gentamicin induced nephrotoxicity in rat.

Key words: Ampicilin, gentamicin, nephrotoxicity, rat, therapy, Iran

INTRODUCTION

Gentamicin a derivative of aminoglycosides is a broad spectrum antibiotic which is widely used for the treatment of gram negative bacterial infections. Its clinical use however is limited due to its side effects such as nephrotoxicity (Pedraza-Chaverri *et al*., 2003). Nephrotoxicity is the major side effect of aminoglycosides, accounting for 10-15% of all cases of acute renal failure (Homes and Weinberg, 1986). The specificity of gentamicin renal toxicity is apparently related to its preferential accumulation in the renal convoluted tubules and its effect on biological membranes (Prayle *et al*., 2010).

Agents which reduce its nephrotoxic effect could help to make gentamicin therapy safer. Many different chemical agents were used to prevent nephrotoxicity in both animal models and human subjects (Dieperink *et al*., 1986; Schrier and Burke, 1988). From the fact that approximately, 90% of ampicillin is eliminated by tubular secretion and 10% by glomerular filtration.

Its concentration in the urine typically will exceed 1000 mcg mL^{-1}. The percentage of ampicillin excreted in the urine appears independent of age and dosage. Penicillins and aminoglycosides are commonly used in combination to treat a variety of infections (Kaplan *et al*., 1974; Weinstein and Moellering, 1973). It is therefore, speculated that the high affinity of ampicillin to the tubular secretion system could protect against the nephrotoxicity due to gentamicin. Present study aimed to study the protective effect of ampicillin on gentamicin induced nephrotoxicity in rat.

MATERIALS AND METHODS

About 40 adult male Wistar rats with body weight ranged from 290-310 g were used in the present study. The rats were kept singly in metabolic cages under conditions of controlled temperature and 12 h day-night cycle, allowed free access to water and fed *ad libitum* a standard rat chow. The rats were randomly divided into four groups of 10 animals each. Group 1 which received only normal saline throughout the course of the experiment was used as control. In group 2, gentamicin was given intramuscularly for 10 consecutive days at the dose of 40 mg kg^{-1}.

Rats in group 3 received 40 mg kg^{-1} gentamicin and 50 mg kg^{-1} ampicillin intramuscularly for successive 10 days. Rats in group 4 were given 50 mg kg^{-1} ampicillin intramuscularly for successive 10 days. About 1 day after

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last injection, rats were weighted and blood was collected by orbital sinus under chloroform anaesthesia for the assay of serum creatinine and Blood Urea Nitrogen (BUN). Serum creatinine and BUN levels, used as markers of renal function were measured by an autoanalyzer system (Beckman Instruments, Calif., USA). The rats were then sacrificed and portions of kidneys from all the four groups were fixed in 10% neutral buffered formalin and processed to paraffin wax. Specimens were sectioned (5 μ). Sections were then stained with haematoxylin and eosin and were examined under light microscope.

They were assessed as described by Houghton *et al.* (1978) as follows: 0 = normal; 1 = areas of focal granulovacoular epithelial cell degeneration and granular debris in tubular lumens with or without evidence of tubular epithelial cell desquamation of small foci (<1% of total tubule population); 2 = tubular epithelial necrosis and desquamation easily seen but involving less than half of cortical tubules; 3 = more than half of proximal tubules showing desquamation of necrosis but involved tubules easily found; 4 = complete or almost complete tubular necrosis.

The significance of differences among the groups was assessed using one way Analysis of Variance (ANOVA) followed by multiple comparison test. The $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

As shown in Fig. 1 although, rats in experimental groups had lower body weight compared with control group. This was significant just in rats receiving gentamicin at a dose of 40 mg kg^{-1} for 10 days ($p<0.05$). Differences in the body weight between experimental groups were not significant ($p>0.05$). As shown in Fig. 2 and 3, marked increases in BUN and serum creatinine levels were observed in n treated group compared to other groups ($p<0.05$). Co-administration of ampicillin decreased the rise in BUN and serum creatinine level. There were no significant differences in BUN and serum creatinine levels between control group compared to groups receiving ampicillin alone or in combination with gentamicin ($p>0.05$). The histological changes in kidney of all the groups were graded and the results are shown in Table 1. The most observed abnormality in rats treated with 40 mg kg^{-1} gentamicin was severe acute tubular necrosis and tubular epithelial loss resulting in tubular cast formation (Fig. 4).

This was often accompanied by tubular hemorrhage, extensive hyperemia and interstitial nephritis. The percentage of damaged proximal tubules in this group was >95%. The histopathology of rats treated with gentamicin

Table 1: Grading of histopathological examination of rat kidney treated with gentamicin and ampicillin

Groups	Grades
Control	0^+
Gentamicin	4^+
Gentamicin+Ampicillin	1^+
Ampicillin	0^+

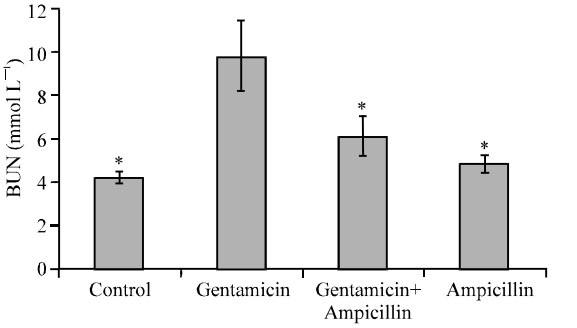

Fig. 2: Effect of gentamicin and ampicillin on BUN. All values are expressed as mean±SD (n = 10) *$p<0.05$ compared to gentamicin group

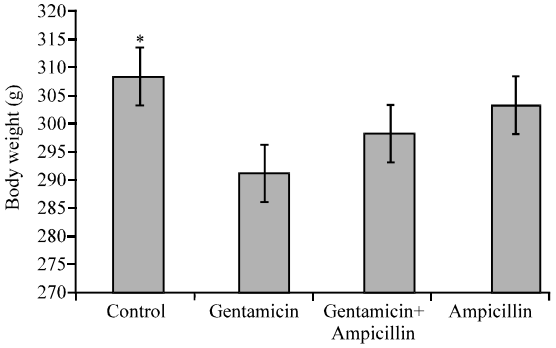

Fig. 1: Effect of gentamicin and ampicillin on body weight. All values are expressed as mean±SD (n = 10); *$p<0.05$ compared to gentamicin group

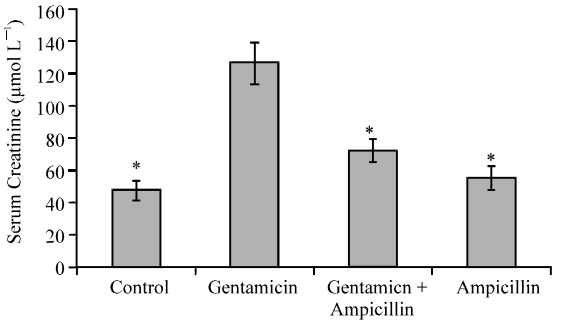

Fig. 3: Effect of gentamicin and ampicillin on serum creatinine. All values are expressed as mean±SD (n = 10) *$p<0.05$ compared to gentamicin group

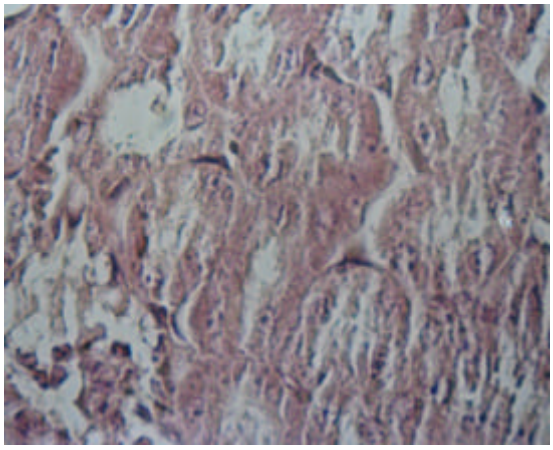

Fig. 4: Acute tubular necrosis in the kidney of rat in gentamicin treated group (H and E, x400)

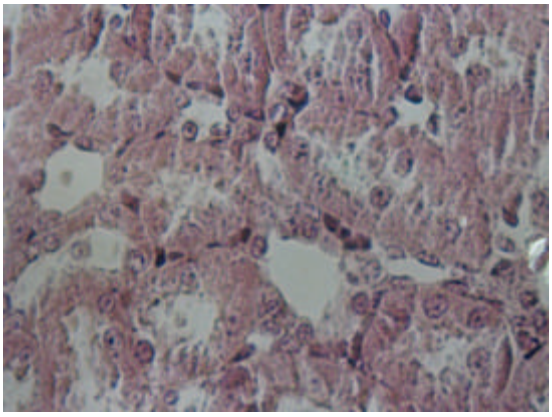

Fig. 5: Mild tubular changes in the kidney of rats treated with gentamicin in combination with ampicillin (H and E, x400)

in combination with ampicillin showed mild tubular changes (Fig. 5). There were not renal histological alterations in control group and in group that received ampicillin alone.

Aminoglycosides are frequently used in combination with penicillins to treat a variety of infections. The use of penicillin or ampicillin in combination with an aminoglycoside has been showed to be advantageous in the treatment of enterococcal infections (Weinstein and Moellering, 1973). Aminoglycosides clinical use however is limited due to their side effects such as nephrotoxicity (Pedraza-Chaverri *et al*., 2003).

Study about aminoglycosides potential nephrotoxicity in combination with penicillins is limited to a few studied on extended-spectrum penicillins such as ticarcillin, carbenicillin and Piperacillin (Sabra and Branch, 1990). In the present study, the researchers the nephrotoxicity of gentamicin either alone or in combination with ampicillin in rats. The kidney function, represented by the serum urea and creatinine concentrations, showed significant impairment following gentamicin treatment at a dose of 40 mg kg^{-1} which was supported by the histopathological examination of the kidney cortices. This was ascribed to the accumulation of the drug in the proximal tubular cells of the kidney cortex and its effect on biological membrane (Prayle *et al*., 2010).

These results were in line with other investigators findings where a significant elevation in the serum urea and creatinine concentrations were demonstrated in rats exposed to 40 mg kg^{-1} gentamicin (Kosek *et al*., 1974; Gilbert *et al*., 1978). However, administration of gentamicin in combination with ampicillin provided marked functional and histological protection against acute renal damage in rats treated with gentamicin. Increase in blood urea and serum creatinine induced by gentamicin was prevented by ampicillin.

Aminoglycosides have no known metabolites and the kidney is virtually entirely responsible for aminoglycoside excretion (Bennett, 1983). The exact mechanism by which aminoglycosides including gentamicin induces renal damage is not fully clear. However, the common understanding is that the development of nephrotoxicity is related to accumulation of aminoglycosides, particularly in the renal proximal tubule cells (El-Mouedden *et al*., 2000; Prayle *et al*., 2010).

Protection against aminoglycoside-induced nephrotoxicity has been demonstrated for many antibiotics or chemical compounds (Kosek *et al*., 1974; Gilbert *et al*., 1978; Dieperink *et al*., 1986; Schrier and Burke, 1988; Sabra and Branch, 1990). This protection occurs with or without a reduction in the uptake of the aminoglycosides, suggesting different mechanisms of protection.

The mechanism by which ampicillin prevent gentamicin induced nephrotoxicity is unknown but from the fact that penicillins including ampicillin have a high affinity for the tubular secretion system and are mainly excreted by tubular secretion. It is therefore, cab be speculated that the high affinity of ampicillin to the tubular secretion system and its high concentrations in renal cortex interfered with or altered the nephrotoxicity due to gentamicin.

The present study suggests that ampicillin protects proximal tubular cells of renal cortex against gentamicin-induced nephrotoxicity. A possible protective effect of ampicillin is inhibition of accumulation of gentamicin in the renal cortex of animals receiving the combination ampicillin-gentamicin compared with animals treated with gentamicin alone.

CONCLUSION

The results of the present study indicate that co-administration of a mpicillin along with gentamicin prevents both functional and histological renal changes induced by gentamicin in rats.

REFERENCES

Bennett, W.M., 1983. Aminoglycoside nephrotoxicity. Nephron, 35: 73-77.

Dieperink, H., P.P. Leyssac, H. Starklint, K.A. Jorgensen and E. Kemp, 1986. Antagonist capacities of nifedipine, captopril, phenoxybenzamine, prostacyclin and indomethacin on cyclosporine-induced impairment of rat renal function. Eur. J. Clin. Invest., 16: 540-548.

El-Mouedden, M., G. Laurent, M.P. Mingeot-Leclercq and P.M. Tulkens, 2000. Gentamicin-induced apoptosis in renal cell lines and embryonic rat fibroblasts. Toxicol. Sci., 56: 229-239.

Gilbert, D.N., C. Plamp, P. Starr, W.M. Bennett, D.C. Houghton and G. Porter, 1978. Comparative nephrotoxicity of gentamicin and tobramycin in rats. Antimicrob. Agents Chemother., 13: 34-40.

Homes, H.D. and J.M. Weinberg, 1986. Toxic Nephropathies. In: Kidney, Brenner, B.M. and F.C. Jr. Rector (Eds.). Saunders Co., Philadelphia, pp: 491-533.

Houghton, D.C., C.E. Plamp, J.M. Defehr, W.M. Bennett, G. Forter and D. Gilbert, 1978. Gentamicin and tobramycin nephrotoxicity. A morphologic and functional comparison in the rat. Am. J. Pathol., 93: 137-152.

Kaplan, J.M., G.H. McCracken, L.J. Horton, M.L. Thomas and N. Davis, 1974. Pharmacologic studies in neonates given large dosages of ampicillin. J. Pediatr., 84: 571-577.

Kosek, J.C., R.I. Mazze and M.J. Cousins, 1974. Nephrotoxicity of gentamicin. Lab. Invset., 30: 48-57.

Pedraza-Chaverri, J., A.E. Gonzalez-Orozco, P.D. Maldonado, D. Barrera, O.N. Medina-Campos and R. Hernandez-Pando, 2003. Diallyl disulfide ameliorates gentamicin-induced oxidative stress and nephropathy in rats. Eur. J. Pharmacol., 473: 71-78.

Prayle, A., A. Watson, H. Fortnum and A. Smyth, 2010. Side effects of aminoglycosides on the kidney, ear and balance in cystic fibrosis. Thorax, 65: 654-658.

Sabra, R. and R.A. Branch, 1990. Role of sodium in protection by extended-spectrum penicillins against tobramycin-induced nephrotoxicity. Antimicrob. Agents Chemother., 34: 1020-1025.

Schrier, R.W. and T.J. Burke, 1988. Calcium-channel blockers in experimental and human acute renal failure. Adv. Nephrol. Necker. Hosp., 17: 287-299.

Weinstein, A.J. and R.C. Jr. Moellering, 1973. Penicillin and gentamicin therapy for enterococcal infections. J. Am. Med. Assco., 223: 1030-1032.