KenKyu

Kenkyu Journal of Pharmacology 4:1-3 (2018)

Research

Effect of Myristic Acid Supplementation on Gentamicin Induced Nephrotoxicity in rats

Anup A.patil[1]*,Rajendra Doijad[2],A.A.Shete[3],A.A.Koparde[4],S.N.Mandrupkar[5]

Professor of KIMSDU'S Krishna Institute of Pharmacy, Malkapur, Karad, India

Received May 15, 2018; **Accepted** July 03, 2018; **Published** July 06,2018

***Corresponding author:** Professor of KIMSDU'S Krishna Institute of Pharmacy, Malkapur, Karad, India, Email: anuppatil.pharma@gmail.com, Mobile: 09096801200.

Abstract

The present study is related to the effects of Myristic acid supplementation on gentamicin induced nephrotoxicity in rats. Three treatment groups (Pretrement, Co-treatment and post treatment) were chosen for the study. Nephrotoxicity in rats was induced by intraperitonial administration of gentamicin (80 mg/kg/d) for 3, 5,7,10, & 12 consecutive days. The animals were sacrificed 12 hrs after last treatment in each group. The maximum nephrotoxicity was developed on 10 days treatment of gentamicin. For each group a control group was taken without any oil or gentamicin treatment. Beneficial effects of myristic acid were evidenced by reduced serum urea and creatinine concentrations in the group receiving myristic acid compared to the non oil treatment animals receiving gentamicin only. Further, the changed values of alkaline phosphatase and acid phosphatase activity returned to normal in kidney and liver tissue homogenates after myristic acid treatment. In this study, it was found that co-treatment of myristic acid is more effective antagonist of gentamicin induced nephrotoxicity. However myristic acid was found to be more effective. Hypercholesteromia associated with gentamicin induced nephrotoxicity is also lowered by myristic acid supplementations. The beneficial effects of these fatty acid are due to counteracting effect of the biochemical alterations induced by the drug.

Key Words: Myristic acid; Nephrotoxocity; gentamycin.

Introduction

Gentamicin is prescribed in life threatening gram–ve infections and its most unavoidable effect is nephrotoxicity despite close attention to the pharmacokinetics and dosing schedule of the drug [1]. Antibiotics have broad antibacterial spectrum ranging from the +ve aerobic cocci to gram–ve. These are clinically stable. It has been reported that 30% of the patients treated with aminoglycosides show some signs of nephrotoxicity like other aminoglycosides gentamincin is eliminated by glomerular filtration but as a result of absorptive endocytosis, gentamicin is partially reabsorbed by proximal tubular cells. Gentamicin loaded endocytic vacoules fuse with lysosomes where the drug accumulates [2, 3]. This accumulation leads to development of lysosomal phospholipidosis characterized by an impairment of phospholipase and sphingomyelinase activities [4]. This phospholipidosis eventually leads to tubular regeneration [5]. It has been reported that the concomitant injection of daptomycin [6] poly-L-aspartic acid and [7] ceftriaxone [8] reduce significantly the renal toxicity of aminoglycosides in experimental animals. Fish oil has also been reported to protect against acetaminophen (paracetamol) induced hepatotoxicity [9], ethanol induced gastric mucosal injury in rats [10] and in a number of inflammatory diseases [11]. In this work different treatment groups pre-treatment, co-treatment, and post-treatment groups have been studied to know the mode of fatty acid treatment that more beneficial in antagonizing gentamicin induced nephrotoxicty

Materials And Methods

Materials

Myristic acid purchased form sigma Aldrich was orally administered (200 mg-body weight) Gentamicin vials of 2ml, having concentration of gentamicin 80 mg in 2ml, obtained from Parkin Remedies, India, were given i.p in one daily dose (80 mg/kg b.wt). All the chemicals used were of the highest purity available commercially and were obtained from E-Marck and Qualigens fine chemicals.

Animals

Male albino rats weighing 100-120gm were used in the experiments. All rats were kept at room temperature 22±2°C in the department animal house. They were maintained on Hindustan Lever food pellets and water ab libitum. The experiment protocol was approved by the Institutional Animal Ethics committee (IAEC). Maximum nephrotoxicity was induced in rats by i.p administration of gentamicin (80mg/Kg/d) for 3, 5, 7,10, and 12 consecutive days. The animals were sacrificed 12h after each injection and serum obtain was subjected to analysis of urea and creatinine to evaluate the nephrotoxicity induced by the antibiotic. Maximum nephrotoxicity was developed after 10 day treatment with gentamicin.

Treatment of Animals

The rats were divided into 5 groups, each having 10 rats. All of these groups were treated as follows: Group C (Control) or no treatment group: The rats were given no treatment at all. They were allowed free access to food and water. Group G or Gentamicin treated group: The animals were given gentamicin (i.p) for last 10 days to get the measure of maximum nephrotoxicity induced by drug. Group I or Pre-Treatment Group: The animals were given orally myristic acid(200 mg) for 10 days and for next 10 days both myristic acid(200 mg) and gentamicin was administered. Group II or Co-Treatment Group: The animals were given no treatment for first 10 days and for next 10 days they were given both myristic acid (200 mg) and gentamicin. Group III or Post-Treatment Group: Here the animals were given gentamicin for first 10 days for next 10 days.

Biochemical Analysis

The animals were given pre, post and co-treatment of either of the myristic acid (200 mg) with gentamic in injections and were sacrificed 12h after they received the last treatment. Blood was withdrawn and serum was obtained by centrifugation of blood at 2000 rpm for 10 minutes. The serum was then deproteinized with 3% TCA in the ratio of 1:3. After incubation for 10 minutes at room temperature the samples were centrifuged at 1500 rpm for 10 minutes to obtain protein free serum, which was subjected to various assays.

1. Quantitative determination of urea by Dam method as described by Fingerhunt et al. [12] using a reagent kit from Techno. Pharm.Chem India.
2. Creatinine estimation was done by method of Taussky and Bonses [13] using a reagent Kit obtained from Span diagnostics Ltd., India.
3. Estimation of cholesterol content by method of Wybenga and Pillegi [14] using reagent Kit from Span diagnostics Ltd , India.
4. SGOT and SGPT levels were determined by method of Reitman and Frankel [15] using a kit obtained from Span diagnostics Ltd, India.

Kidney and Liver Homogenates

Kidney and liver was removed rapidly and were homogenized separately in Mannitol (50 mM) using a high-speed Turrex homogenizer. Supernatant was obtained by centrifugation of homogenate at 4°C for 10 minutes at speed of 20,000 rpm. Supernatant was then subjected to assay of marker enzymes.

1. Alkaline phosphatase assay The activity of AlP was determined according to the method of Shah et al. [16].
2. Acid phosphatase assay The AcP activity was measured quantitatively by the method of Verjee [17].

Statistical Analysis

Present data are Mean ± SEM for at least four separate experiments. Statistical analysis of the data was performed using by student's t-test (20) $p < 0.05$ and $p < 0.01$ are considered significant.

Results

In the present study, Gentamicin, a nephrotoxic aminoglycoside was injected in adult male. The impaired renal function is reflected by increased urea and Creatinine in the serum of gentamicin receiving group compared to control rats. The rise in levels of other serum parameters like cholesterol, serum glutamic oxaloacetic transminase (SGOT), serum glutamic pyruvic transminase (SGPT), as well as decrease in activities of various marker enzymes like AlP and AcP in liver and kidney homogenates suggest that gentamicin is toxic for various organs. From the table 1 it is clear that gentamicin treatment results in increase in serum urea concentration. This increased level was found to return towards normal in co-treatment of Myristic acid. Pre and post treatment of the myristic acid. however, did not bring pronounced effect. Similarly, the elevated levels of creatinine in serum by gentamicin treatment were again found to be normalized in co-treatment myristic acid. groups. treated groups.

Gentamicin causes hypercholesterolemia as is evident by the rise in level of cholesterol in gentamicin treated group. Animals fed on mristic acid had significantly lower cholesterol. The serum SGOT and SGPT levels measured show significant increase in gentamicin treated animal groups Co-treatment of myristic acid was found to bring these high levels of SGOT and SGPT back towards normal.

The effect of gentamicin was also observed in the activities of certain enzymes of kidney and liver. The AlP and AcP level was found to change significantly in the homogenates on treatment with gentamicin injections. In kidney homogenate, the level of AlP was found to be significantly lowered in post and co-treated myristic acid groups, where as the level of AcP was normalized significantly in co-treated myristic acid groups. Further, table-2 gives the levels of AlP and AcP in liver homogenates. These changed levels of AlP and Acp returned towards normal value in co-treatment myristic acid group.

Groups	Urea mg/100mg	Creatinine mg/100mg	Cholesterol mg/100mg	SGOT nmole/ min/ml	SGPT nmole/ min/ml
Control	5.031±0.51	0.854±0.05	107.89±3.69	25.63±0.220	30.06±0.158
Gentamycin	11.85±1.14	1.26±0.07	125.43±0.489	90.90±0.450	94.57±0.446
Pre-Myrisitic acid	11.16±0.56	1.18±0.69	114.25±1.32[A]	30.36±0.355[A]	32.76±0.299 [A]
Co-Myristic acid	6.51±0.323[A]	1.099±0.052	113.26±2.93[A]	28.25±0.705 [A]	30.76±0.299 [A]
Post-Myrisitc acid	10.89±0.92	1.253±0.052	112.56±1.56[A]	50.30±0.274	43.54±0.122
AP<0.05 as compared to control rats.					

Table 1: Circulating levels of various biochemical parameters in serum.

Groups	Kidney A/P nmole/mg/ml	Kidney Acp nmole/min/ml	Liver A/P nomle/min/ml	Liver Acp nomle/min/ml
Control	49.41±0.885	70.56±3.58	2.56±0.830	138.5±12.97
Gentamycin	40.22±5.64	141.82±25.89	1.73±0.184	85.15±9.43
Pre-Myrisitic acid	44.44±2.61	102.02±13.15	1.57±0.14	120.23±7.85
Co-Myristic acid	45.29±3.11[A]	79.30±1.29 [A]	2.29±0.301	121.11±1.709
Post-Myrisitc acid	40.57±1.80[A]	96.28±16.03 [A]	1.94±0.052[A]	92.58±2.07
[A]P<0.05 as compared to control rats.				

Table 2 : Alkaline and Acids Phosphatase levels in kidney and homogenates.

Discussion

The beneficial effects of myristic acid are due to their constituent fatty acids. The protective effects of n- 3 fatty acids, eicosapentaenoic and decosahexaenoic acid present have been established in cyclosporine treated patients and nephrotoxic experimental animals [18]. It has been reported that fish oil (fatty acid) reduces circulating lipid levels in animals as well as in human subjects [19].

Acute renal failure, induced in animals by gentamicin exposure was manifested by increased serum urea and creatinine levels. The rise of urea and Creatinine levels in gentamicin treated animals [20] compared to control treated animals suggest that due to renal injury, glomerular filtration rate (GFR) and reabsorbtion processes have been effected [21]. From the results it can be concluded that both myristic acid are effective antagonists of gentamicin induced nephrotoxicity. In addition our studies suggest that myristic acid is more useful than other treatment groups studied the maximum recovery effect was observed in co-treatment groups. The data presented in this manuscript could be helpful in formulating human clinical trials to examine the efficacy of myristic acid supplementation on nephrotoxicity induced by commonly used antibiotics

Conclusion

The present study is a comparative study in which the object was to investigate the beneficial effects of myristic acid on gentamicin induced nephrotoxicity in rats. Results indicate that these myristic acid alter the levels of enzymes studied and bring them back to control levels. Therefore, we conclude that myristic acid compounds prescribed as co-treatment with drug so as to reduce gentamicin side effects including nephrotoxicity with out compromising its antibiotic activity.

References

1. Lee S. M, Pattison M.E, Michael U. F (1987) Nitrendipine protects against aminoglycosid nephrotoxicity in rats. J. of cardiovascular. Pharmacol 9: 65-69.
2. Beauchamp D, Gourde, Bergeron M. G (1991) Subcellular distribution of gentamycin in proximal tubular cells, determined by immunogold labeling. Antimicrobial Agents and Chemotherapy 35: 2173-2179.
3. Silverblatt F. J , Knehn C (1979) Autobiography of gentamycin uptake by the rat proximal tubule cells. Kidney Int 15: 335–345.
4. Laurent G M. B, Carlier B, Rollmann F, Vantthoff P.M, Tulkens (1982) Mechanism of aminoglycoside induced lysosomal phospholipidosis in vitro and in vivo studies with gentamicin and amikacin. Biochem. Pharmacol 31: 3861–3870.
5. Ginliano R.A, G.J. Paulus, G.A. Verpooten, V. Pattyn , D. E. Pollet et al. (1984) Recovery of cortical phospholipidosis and necrosis after acute gentamicin loading in rats. Kidney Int. 26: 838 – 847.
6. Beauchamp D, M. Pellerin, P.Gourde, M.Pettigrew, M. G. Bergeron (1990) Effects of daptomycin and vancomycin on tobramycin nephrotoxicity in rats. Antimicrobial. Ag.Chemotherapy 34:139–147.
7. Beauchamp D, G. Laurent, P. Maldagne, S. Abid, B.K.Kishore et al. (1990) Protection against gentamicin induced early renal alterations (Phospholipidosis and increased DNA synthesis) by co-administration of poly – L – aspartic acid. J. of Pharmacol. Exp. Therapy 255: 858 – 866.
8. Beauchamp .D, G. Theriault, L. Grenier , P. Gourde, S. Perron et al. (1994) Ceftriaxone protects against tobramicin nephrotoxicity. Antimicrobial Ag. Chemotherapy 38:750–756.
9. Speck R.F, Lauterberg B. H (1999) Fish oil protects mice against acetaminophen hepatotoxicity in vivo. Hepatology 13:557– 561.
10. Leung F.W (1992) Fish oil protects against thanol – induced gastric mucosal injury in rats Digestive Diseases and Sciences. 37: 636 – 637.
11. Clark W. F, Parbtani .A, Naylor. D, Levinton.C , Muirhead. N et al. (1993) Fish oil in upus nephritis – clinical findings and methodological Implications. Kidney Int 44:75–76.
12. Fingerhunt B, Ferzola R , Marsh W.H , Miller A (1966) Automated methods for blood glucose and urea with adaptation for simultaneous determination in. Chem 9: 570-576.
13. Bonses R.M, Taussky H.H (1945) On olorimetric determination of creatinine by Jaffe reaction. J. Biol. Chem. 158:581-591.
14. Wyben D. R, Pillegi V. J , Dirstine D. H ,Digiorgio J (1970) Direct manual determination of serum total cholesterol with a single stablereagent. Clin. Chem 16: 980-984.
15. Reitman S, Frankel S (1975) Colorimetric method for the dterminatiion serum glutamic oxaloacetic and gutamic transaminases. Am. J. clin Path. 28: 56-63.
16. Shah S. V, Kempson S. A, North Rap T. E, Dousa T. P (1979) Renal adaptation to a low phosphate diet in rats. J. Clin. Investigation 64:955-966.
17. Verjee Z.H.M (1969) Isolation of three acid phosphatases from wheat germ. Eur. J. of Biochem. 9:439–444.
18. Morio saito, Kazuhiro Kubo (2003) Relationship between tissue lipid per oxidation and peroxidizability index after linolinic, eicosapentaenoic, or docosahexaonoic acid intake in rats. Br. J. Nutr 89: 19-28.
19. Connor W.E (2000) Importance of n-3 fatty acids in health and disease. Am. J. Clin. Nutr. 71: 171-175.
20. Lietman P. S, Smith C. R (1983) Aminoglycoside nephrotoxicity in humans. Rev. Infect. 5:284-289.
21. Neugarten F, Aunedjian H. S , Bank N (1983) Role of tubular obstruction in acute renal failure due to gentamycin. Kidney Int 24: 330-335.