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COMPARATIVE EFFECT OF OLIVE OIL AND FISH OIL SUPPLEMENTATION IN COMBATING GENTAMICIN INDUCED NEPHROTOXICITY IN RATS

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ABSTRACT

The present study is related to the comparative effects of fish oil and olive oil 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 oils were evidenced by reduced serum urea and creatinine concentrations in the group receiving oils 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 fish and olive oil treatment. In this study, it was found that co-treatment of fish and olive oil is more effective antagonist of gentamicin induced nephrotoxicity. However fish oil was found to be more effective. Hypercholesteromia associated with gentamicin induced nephrotoxicity is also lowered by oil supplementations. The beneficial effects of these oils are due to counteracting effect of the biochemical alterations induced by the drug.

KEYWORDS

Cholesterol, Creatinine, Fish oil, Gentamicin, Kidney, Nephrotoxicity, Olive oil, Urea.

INTRODUCTION

Antibiotics have achieved widespread clinical use as effective antimicrobial drugs. These are clinically stable and rapid in their action. Antibiotics like gentamicin, amikacin and tobramicin are aminoglycosides in nature, having sugar in glycoside linkage with side chains containing amino groups. They are highly charged and water soluble at physiological pH. The commonly used clinically stable aminoglycosides like gentamicin have free amino groups and thus the net cationic charge correlates well with the nephrotoxicity of these compounds. 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 bacilli. It has been reported that 30% of the patients treated with aminoglycosides show some signs of nephrotoxicity (2). 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 (3, 4). This accumulation leads to development of lysosomal phospholipidosis characterized by an impairment of phospholipase and sphingomyelinase activities (5). This phospholipidosis eventually leads to tubular regeneration (6). It has been reported that the concomitant injection of daptomycin (7) poly-L-aspartic acid and (8) ceftriaxone (9) reduce significantly the renal toxicity of aminoglycosides in experimental animals. Fish oil has also been reported to protect against acetaminophen (paracetamol) induced hepatotoxicity (10), ethanol induced gastric mucosal injury in rats (11) and in a number of inflammatory diseases (12).

Recently it has been shown that fish oil supplementation of gentamicin induced nephrotoxicity leads to beneficial effects on the kidney of these rats (13). Thus it was thought worthwhile to investigate in detail the supplementation of fish oil and olive oil as well as to study the comparative effect of these two oils

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in prevention of gentamicin induced nephrotoxicity. In this work different treatment groups pre-treatment, co-treatment and post-treatment groups have been studied to know the mode of oil treatment that is more beneficial in antagonizing gentamicin induced nephrotoxicity.

MATERIALS AND METHODS

Materials:

Fish oil from Seven Sea Ltd., UK and Olive oil from Milano, Italy was orally administered (5ml/kg-body weight) with the help of catheter to rats. 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) of the University.

Time dependent effect of Gentamicin in Rats:

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 8 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 Oil Pre-Treatment Group: The animals were given oil orally for 10 days and for next 10 days both oil and gentamicin was administered.

Group II or Oil Co-Treatment Group: The animals were given no treatment for first 10 days and for next 10 days they were given both oil and gentamicin.

Group III or Oil Post-Treatment Group: Here the animals were given gentamicin for first 10 days and oil for next 10 days.

Group I, II, and III were in duplicates, one set being given fish oil and other olive oil.

Biochemical analysis:

The animals were given pre, post and co-treatment of either of the oils with gentamicin 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. (14) using a reagent kit from Techno. Pharm. Chem; India.
2. Creatinine estimation was done by method of Taussky and Bonses (15) using a reagent Kit obtained from Span diagnostics Ltd., India.
3. Estimation of cholesterol content by method of Wybenga and Pillegi (16) using reagent Kit from Span diagnostics Ltd., India.
4. SGOT and SGPT levels were determined by method of Reitman and Frankel (17) 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 (AlP, E.C.3.1.3.1): The activity of AlP was determined according to the method of Shah et al. (18).
2. Acid phosphatase assay (AcP, E.C.3.1.3.2): The AcP activity was measured quantitatively by the method of Verjee (19).

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 rats alone or together with fish oil or olive oil. 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 fish and olive oil groups. Pre and post treatment of the two oils 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 fish and olive oil groups.

Gentamicin causes hypercholesterolemia as is evident by the rise in level of cholesterol in gentamicin treated group. Animals fed on n-3 fatty acids (fish oil) had significantly lower cholesterol content compared to those fed on n-6 fatty acids diet (olive oil) as is suggested in table-1. The serum SGOT and SGPT levels measured show significant increase in gentamicin treated animal groups Co-treatment of fish oil was found to bring these high levels of SGOT and SGPT back towards normal compared to all other oil treated groups.

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 oil groups, where as the level of AcP was normalized significantly in co-treated oil 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 fish and olive oil groups.

DISCUSSION

The beneficial effects of fish oil and olive oil are due to their constituent fatty acids. The protective effects of n-3 fatty acids, eicosapentaenoic and decosahexaenoic acid present in fish oil have been established in cyclosporine treated patients and nephrotoxic experimental animals (9, 21). It has been reported that fish oil reduces circulating lipid levels in animals as well as in human subjects (22). In earlier studies (13) it was reported that fish oil has beneficial effect on gentamicin induced nephrotoxicity but our results show that besides fish oil, olive oil also has role to play in combating gentamicin induced nephrotoxicity.

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 (23) compared to control treated animals suggest that due to renal injury, glomerular filtration rate (GFR) and reabsorbtion processes have been effected (24). Our study indicates the beneficial effects of fish and olive oil in combating nephrotoxicity, as the levels of urea and creatinine decreased from higher levels in co-treatment both oil treated groups.

To see any cholesterol lowering effects, the level of cholesterol was determined and it was found that pre, co and post fish oil treatment resulted in significant decreased by gentamicin alone (Table-1). Co-treatment with olive oil was also found to lower cholesterol to same extent when compared to normal. In plasma, the lower cholesterol level in the fish oil consuming animals has been reported by Valentina and Alonso (25). In accordance with this, our comparative study shows that fish oil is better than olive oil in lowering the cholesterol level. Many clinical studies have indicated that diets rich in fish oil are associated with cardiovascular health and the responsible component was found to be high content of PUFA of the n-3 series (26-28). Olive oil, an oil rich in MUFA is also related to cardiovascular health. It has been found that besides oleic acid, sterols and polyphenols in olive oil may contribute to these beneficial results (29,30).

Authors have already reported that SGOT level increases in patients receiving antibiotic injections (31). This was in accordance with our observations, where animals receiving gentamicin injections were found to have elevated levels of SGOT and SGPT thereby indicating that liver damage may have occurred to some extent. Oral administration of fish and olive oil after gentamicin injections helped in lowering the levels of SGOT and SGPT, which otherwise were increased by gentamicin alone. The decreased activities of AlP and AcP in kidney and liver homogenates returned back maximally towards normal in co-treatment fish and olive oil treated animals, indicating the beneficial use of fish and olive oil on gentamicin exposure.

From the results it can be concluded that both olive oil and fish oil are effective antagonists of gentamicin induced nephrotoxicity. In addition our studies suggest that fish oil is more useful than olive oil and among different 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 fish and olive oil supplementation on nephrotoxicity induced by commonly used antibiotics

Table 1. Circulating levels of various biochemical parameters in serum

Groups	Control	Gentamicin	Pre-fish oil	Co-fish oil	Post-fish oil	Pre-olive oil	Co- olive oil	Post olive oil
Urea mg/100ml	5.032±0.52	11.95±1.16	11.18±0.606	6.53±0.323[a]	11.63±0.93[a]	6.91±0.78[a]	6.00±0.38[a]	10.9±1.125
Creatinine mg/100ml	0.954±0.05	1.25±0.07	1.18±0.69[c]	1.077±0.052[d]	1.224±0.089	1.091±0.164[a]	0.975±0.164[b]	1.243±0.096
Cholesterol mg/100ml	107.88±3.68	125.43±0.486	114.25±1.32[a]	113.09±2.96[a]	112.38±1.56[a]	122.08±2.50	109.24±2.59[a]	120.61±0.905
SGOT nmol/min/ml	25.69±0.220	90.90±0.451	30.38±0.355[a]	28.25±0.705[a]	50.30±0.274	55.49±0.194	31.26±0.302[a]	61.06±0.315
SGPT nmol/min/ml	30.07±0.157	94.57±0.443	32.16±0.299[a]	30.76±0.137[a]	43.54±0.122	40.94±0.167	35.05±0.329[a]	52.35±0.535

[a]P<0.05 [b]P<0.02 [c]P< 0.01 [d]P< 0.001 as compared to control rats.

Table 2. Alkaline and Acid Phosphatase levels in kidney and liver homogenates

Groups	Control	Gentamicin	Pre-fish oil	Co-fish oil	Post-fish oil	Pre-olive oil	Co- olive oil	Post olive oil
Kidney Alp								
nmol/mg/ml	49.41+0.885	40.22±5.64	44.44+2.61	45.29±3.11[a]	40.57±1.80[a]	45.20±4.01[a]	48.35±1.689[a]	41.03±1.43
Kidney Acp								
nmol/min/ml	71.66±3.58	141.82±26.47[a]	102.02±13.15	79.66±2.03[d]	129.30±1.30	96.28±16.03[a]	73.26±2.57[b]	112.37±12.37
Liver Alp nmol/min/ml	2.65±0.830	1.73±0.184	1.57±0.14[b]	2.29±0.301[a]	1.94±0.052[a]	2.94±0.218	2.61±0.218[a]	2.05±0.62
Liver Acp								
nmol/min/ml	138.5±12.97	85.15±9.43	120.23±7.85[a]	121.11±1.709[a]	92.58±2.07[b]	128.88±11.47	120.54±1.108[a]	98.82±13.39[b]

[a]P<0.05 [b]P<0.02 [c]P< 0.01 [d]P< 0.001 as compared to control rats.

i.e. aminoglycosides.

CONCLUSION

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

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