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MODULATORY EFFECTS OF FISH OIL ON AMIKACIN TOXICITY: INFLUENCES ON ANTIOXIDANT STATUS

МОДУЛЯТОРНЫЕ ЭФФЕКТЫ МАСЛА РЫБЫ НА ТОКСИЧНОСТЬ АМИКАСИНА: ВЛИЯНИЯ НА АНТИОКИСЛИТЕЛЬНЫЙ СТАТУС

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This study was undertaken to investigate the effect of fish oil in reversal of the nephrotoxicity induced by amikacin in rat model. The study was also extended to compare the preventive effects of fish oil in ameliorating amikacin nephrotoxicity to that of olive oil. Animals were given amikacin via intraperitonial route (i.p) at a dose of 250 mg/kg body weight/ day for 8 days to induce maximum nephrotoxicity, which lead to a significant increase in serum urea and creatinine levels. Fish and olive oil was given to these nephrotoxic rats in a pre, post and co treatment for 8 days at the dose of 5 ml/ kg body weight/ day. Pre and post fish oil treatment resulted in normalizing the otherwise elevated levels of urea, creatinine, cholesterol, serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT) and inorganic phosphate. The indices of renal damage were also detected by evaluating the levels of various kidney marker enzymes. Amikacin treatment resulted in a significant fall in levels of alkaline phosphatase (AlP) and acid phosphatase (AcP). The decreased level of these renal enzymes were also significantly increased with prefish oil treatment. Experimental evidences also suggest a role of reactive oxygen species in antibiotic induced toxicity. Therefore the levels of catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferase (GST) were also determined. Amikacin alone resulted in a significant decrease in the levels of these enzymes. However, feeding fish oil resulted in a significant increase in the levels of these enzymes thereby enhancing the efficiency of the antioxidant defence system. We conclude that fish oil supplementation has a beneficial role in combating the toxicity induced by amikacin, which may be due to the changes in kidney and liver brought about by n-3 fatty acids which are present predominantly in the fish oil. It is established, that liquid crystal membrane systems are exposed to updating.

Исследование было предпринято, чтобы исследовать эффект масла рыбы в предотвращении гипертоксичности, вызываемой амикасином (amikacin) у крыс. Исследование было проведено, чтобы сравнить профилактические эффекты масла рыбы в присутствии оливкового масла. Животным давали амикасин in vivo в дозе 250 мг/кг массы тела в день в течение 8 дней, чтобы вызвать максимальный эффект, который приводит к существенному увеличению мочевины сыворотки и уровня креатинина. Рыба и оливковое масло давались этим крысам с интоксикацией перед, во время и после введения амитаксина в течение 8 дней в дозе 5 мл /кг массы тела /в день. Введение

в рацион рыбы приводило к нормализации повышенных уровней мочевины, креатинина, холестерина, сывороточной глутамат оксалоацетас трансаминазы (SGOT), сывороточной глутамат пируват трансаминазы (SGPT) и неорганического фосфата. Индексы почечного повреждения были также выведены из оценок уровней маркёров различных почечных ферментов. Экспериментально показана роль реактивных разновидностей кислорода в антибиотике в проявлении токсичности. Таким образом, введение в рацион масла рыбы, содержащего ненасыщенные жирные кислоты, играет положительную роль в борьбе с токсичностью, вызванной амитаксином, которая может произойти из-за изменений в почках и печени. Установлено, что происходит модификация жидкокристаллических систем мембран.

Key words: *fish oil, olive oil, amikacin, urea, creatinine, antioxidant enzymes.*

Ключевые слова: *масло рыбы, оливковое масло, амикасин, мочевина, креатини, антиокислительные ферменты*.

Introduction

Aminoglycosides of which amikacin is a prototype, are useful antimicrobial agents. Unfortunately they all possess nephrotoxic properties and acute deterioration in renal function is a serious complication and often a limiting factor to the use of these drugs. Amikacin is a semi synthetic aminocyclitol, and used for treatment of pulmonary, infection, urinary tract infections, septicemia and mycobacterial infection [1] morphological changes of aminoglycoside nephrotoxicity are mainly characterized by reduced renal excretory capacity and by proximal tubule necrosis [2] like gentamicin, it enters renal proximal tubule through pinocytosis and is then sequestered in lysosomes thereby influencing renal tubule metabolism and functions in a variety of ways including alteration in the function of mitochondrial respiration [3]. Amikacin is eliminated from the kidney by glomerular filteration, however, some deposition in cortex occurs which appears to be related to its nephrotoxicity [4]. The nephrotoxic potential of amikacin appears to the same as gentamicin, with initial renal manifestation of toxicity as enzymuria. Further tubular transport can also deteriorate leading to polyuria and nephrogenic diabetes insipidus [5]. Aminoglycosides are also known to inhibit phospholipase in membranes, thereby affecting the activities of various enzymes modulated by phospholipids [6]. A number of agents have been identified as potential factors that may increase or decrease this induced nephrotoxicity [7].

Omega-3 polyunsaturated fatty acids, present mainly in seafood and therefore better known as «fish oil», extert a number of biological effects and there are currently being tested in number of clinical situations. Fish oil enriched diets are helpful in lowering cholesterol levels besides acting as anti-inflammatory and anti-thrombotic agents [8] and the responsible component appears to be eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) [9]. Some vegetable oils like olive oil with oleic acid as mainly fatty acid component have also been related to hypercholesterolemia [10]. There is evidence that olive oil inhibits platelet function and thromboxane synthesis [11]. Evidences are accumulating that suggest the efficacy and potential clinical utility of fish oil in renal diseases [12, 13].Since, morphological changes of aminoglycoside nephrotoxicity are primarily the consequences of damage to cell membrane of which fatty acids remain essential constituents, therefore investigating the effect of these dietary oils on toxicity induced by amikacin becomes important. In view of this the present study was undertaken to see the comparative effect of fish and olive oil on amikacin induced toxicity.

Materials and method

Adult male wistar rats weighing 100 – 150 g were used in all the experiments. Animals were stabilized for 8 days prior to the experiment on standard pellet rat diet, the composition of which is as: Crude protein 21 %, ether extract 5 %, crude fibre 4 %, ash 8 %, calcium 1 %, phosphorus 0,6 %, nitrogen free extract 55 %, Starch 7 %, all vitamins, minerals and trace elements (table 1). The animals were also allowed free access to water. The protocols for the experiments were approved by Institute of Animal Ethical Committee IAEC. Guidelines laid by university committee and principles of laboratory animal care were also followed**.**

Table 1

Fatty acid composition of the oils (g/100 g total fatty acids)

Fatty Acids	FO	OO
14:0	3,8	–
16:0	13,9	11,8
16:1n-7	15,1	0,9
17:0	–	0,4
18:0	0,9	2,8
18:1n-9	26,2	79,2
18:2n-6	5,1	3,5
18:3n-3	0,2	0,6
18:4n-3	2,5	–
20:0	–	0,3
20:1n-9	2,4	0,2
20:2n-6	1,4	–
20:3n-3	0,4	–
20:4n-6	0,6	–
20:5n-3	13,6	–
22:4n-6	0,3	–
22:6n-3	12,7	–
24:0	–	0,4

FO – fish oil, OO – olive oil

Materials

All the chemicals used for the study of biochemical parameters of serum and homogenate, were of analytical grade obtained from commercial sources. Fish oil from Seven Sea Ltd., UK and Olive oil from Milano, Italy was orally administered to rats (5 ml/kg body weight/day) with the help of catheter. The fatty acid composition of the two oils [14] is given in table 1. Amikacin vials of 2 ml, having concentration of amikacin as 80 mg in 2 ml, obtained from Aristo Pharmaceuticals, India, were given intraperitonially (i.p) in one daily dose (250 mg/kg body weight) in volume adjusted to 1 ml with 0,9 % saline for 8 days.

Time dependent effect of amikacin in rats

Maximum nephrotoxicity was induced in rats by intraperitonial administration of amikacin (250 mg/kg body weight/day) for 4,6,8,12,14 consecutive days. The animals were sacrificed 12h after each injection and the serum obtain was subjected to analysis of urea and creatinine to evaluate the nephrotoxicity induced by the antibiotic. Maximum nephrotoxicity was developed after 8 days of treatment with amikacin (table 2).

Table 2

Effect of Amikacin on levels of urea and creatinine

Parameters determined	Days of the experiment					
	0	4	6	8	12	14
Urea mg/100 mL	4,98±0,017	6,31±0,031	8,04±0,160	10,32 [a] ±0,09	10,15±0,217	9,56±0,171
Creatinine mg/100mL	1,12±0,160	2,30±0,099	2,96±0,121	3,97 [a] ±0,059	3,73±0,097	3,45±0,192

Values represent Mean ± SE; n=8, [a] $p<0,05$ with respect zero day treated rats. Rats were sacrificed after 12h after each injection of the drug.

Treatment of animals

The rats were divided into 8 groups, each having 8 rats. All of these groups were treated as follows:

Group I (Normal) or no treatment group. The rats were given no treatment for the first 8 days and intraperitonial injection of normal saline for the last 8 days of the 16 day experiment. Animals were allowed free access to food and water.

Group II or amikacin treated group. The animals were given no treatment for the first 8 days and intraperitonial injection of amikacin for last 8 days of the 16 day experiment to get the measure of maximum nephrotoxicity induced.

Group III or Fish Oil Pre-Treatment Group. The animals were given fish oil orally for 8 days and for next 8 days amikacin was administered.

Group IV or Fish Oil Co-Administration Group. The animals were given no treatment for first 8 days and for next 8 days they were given both fish oil and amikacin.

Group V or Fish Oil Post-Treatment Group. Here the animals were given amikacin for first 8 days and fish oil for next 8 days.

Group VI, VII and VIII were duplicates of group III, IV and V except that rats in these groups given olive oil. The diet intake was same in all groups.

Biochemical analysis

The animals were given amikacin in pre, post and co-administration with either of the oils and were sacrificed 12h after receiving 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 tem-

perature, 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 Marsh WH et al. [15] using a reagent kit from Techno. Pharm. Chem; India.
2. Creatinine estimation was done by method of Tausky and Bonses [16] using a reagent Kit obtained from Span diagnostics Ltd., India.
3. Estimation of cholesterol content by method of Wybenga and Pillegi [17] using reagent Kit from Span diagnostics Ltd., India.
4. Quantitative determination of inorganic phosphate by the method of Tausky and Shorr [18].
5. SGOT (E.C.2.6.1.1) and SGPT (E.C.1.1.1.1) levels were determined by method of Reitman and Frankel [19] using a kit obtained from Span diagnostics Ltd., India.

Kidney and liver homogentates

Kidney and liver were removed rapidly and were homogenized separately in Mannitol (50 mM) using a high-speed turrex kunkel 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 the various enzymes.

1. Alkaline phosphatase assay (Alkpase, E.C.3.1.3.1): The activity of AlP was determined according to the method of Shah et al. [20].
2. Acid phosphatase assay (AcPase, E.C.3.1.3.2): The AcP activity was measured quantitatively by the method of Verjee [21].
3. Superoxide dismutase (E.C.1.15.1.1) in liver homogenate was determined spectrophotometrically by the method of Marklund and Marklund [22].
4. Catalase (E.C.1.11.1.6) in liver homogenate was quantitatively estimated by the method of Beers et al. [23].
5. Glutathione-S-transferase (E.C.2.5.1.18) in liver homogenate was determined by the method of Habig et al. [24].
6. The level of lipid peroxides formed in liver was determined by the method of HalliWell B et al. [25].
7. Protein was estimated in all serum, and tissue samples by the method of Lowry et al. [26].

Statistical analysis

Statistical analysis of the data was performed using one way analysis of variance (ANOVA) using SPSS computer software to compare the means between the different treatment groups and normals. The differences were considered significant when $p<0,05$. Values shown as means ±SE for 8 animals.

Results

In the present study the toxic effects of amikacin and its removal with use of fish and olive oil were studied.

General parameters. The diet intake was the same in all the rat groups. The body weights of different rat groups before and after the completion of experiment was measured. Amikacin treatment alone did not produce any significant change in the overall weight of

animals. Fish and olive administration also did not bring any profound changes in total body weight and weight of kidney and liver of different treated groups

Serum parameters. The determination of levels of various serum metabolites gave the indices of damage caused by amikacin to various organs. Amikacin is nephrotoxic, as is reflected by two fold increase in urea and creatinine levels in rat group receiving amikacin only. However fish oil has a pronounced effect on various serum parameters studied. Fish oil treatment for 8 days prior to and together with amikacin helped in reversion of amikacin induced nephorotoxicity as is given by normalized urea and creatinine levels in these two groups. Among olive oil treated groups, pre olive oil treatment brought the maximum lowering of these increased levels of urea and creatinine($p<0,05$). However the fact remains that compared to fish oil, the effect is much less ($p<0,05$).

Pre fish oil treatment also lowers the otherwise elevated cholesterol levels by amikacin. This hypocholesterolemic effect was again more pronounced in fish oil than olive oil treated groups ($p<0,05$). Amikacin is toxic to liver also, as is evident by increased circulating levels of SGOT, SGPT and inorganic phosphorous. Further gives that maximum reduction in elevated levels of above given parameters, was done by pre fish treatment, suggesting the beneficial role of fish oil in reverting the induced hepatoxicity.

Effects on renal function parameters. The deleterious effects of amikacin on kidney function were detected by determining the activities of various renal marker enzymes. Amikacin injections reduced the levels of AlP and AcP to half indicating that the drug damages renal membrane. However, the activity of these enzymes was restored to normal in pre and post fish oil treated groups. Again among the two given oils, fish oil exerts maximum beneficial effect. The effect of fish oil and olive oil on kidney antioxidant status is given again. Amikacin treatment results in decrease in the levels of CAT, SOD and GST. Fish oil administration resulted in significant increase in the levels of antioxidant enzymes to combat the induced stress by amikacin injections. Among the olive oil groups, preolive oil treatment resulted in increasing the levels of CAT, SOD and GST maximally.

Oxidative stress biomarkers in liver. In order to investigate the effect of fish oil and olive oil on liver antioxidant status, the activities of various antioxidant enzymes were determined. Amikacin induces oxidative stress as is evident by decreased levels of CAT, SOD and GST as compared to non-stressed normal saline treated rats. Rats fed on fish oil exhibited significant higher activities of CAT, SOD and GST compared to amikacin treated rats ($p<0,05$). However olive oil treated rats gave no such significant rise in activities of these enzymes when compared to normal saline treated rat group($p<0,05$). Lipid peroxidation level was also measured. Malondialdehyde (MDA) an end product of lipid peroxidation was found to be elevated in nephrotoxic rats. Further fish oil fed animal groups gave higher levels of MDA as compared to olive oil receiving group.

Discussion

Amikacin is toxic to various organs, therefore identification of different factors that modulate the toxicity of this aminoglycoside is of some clinical significance. In the present study, the effect of fish oil and olive oil on amikacin induced organ toxicity as well as on oxidative stress was evaluated. The results indicated the manifestation of nephrotoxicity by amikacin, by increased serum urea and creatinine concentrations compared to normal saline treated rats. Thus indicating that renal injury, glomerular filteration and reabsorbtion processes have been affected with use of amikacin [27]. The supplementation of rats with fish oil at dose of 5 ml /kg body weight/ day substantially ameliorated the renal alterations associated

with amikacin nephrotoxicity (table 1). This is in accordance with those reported by Ali and Bashir for gentamicin nephrotoxicity [12]. Vast studies have been done on gentamicin, an aminoglycoside, induced nephrotoxicity. Gentamicin induced nephrotoxicity has been associated with increased urinary excretion of TxB_2, a cyclooxygenase metabolite (vasoconstrictor). Further, use of enzymes inhibitors to block the thromboxane A synthase was found to cause improvement in renal functions [28]. In view of above report, the beneficial effect of fish oil, rich in omega fatty acids, against amikacin induced nephrotoxicity, which is structurally related to gentamicin observed in the present study, could possibly be through repressing the renal production of the potent vasoconstrictive and inflammatory series of prostaglandins (thromboxane A_2 and B_2) from arachidonic Acid (AA), an ω-6 fatty acid. This has been suggested to occur by displacement of AA in the phospholipids of glomerular membrane by the ω-3 fatty acids resulting in production of non-inflammatory series 3-thromboxanes and series 5- leukotrienes [29].

Cholesetrol estimation made it clear that amikacin is hypocholesterolemic. Secondary hypocholesterolemia is an established fact for gentamicin nephrotoxicity[30]. This is again accordance with our study, where cholesterol levels were restored to normal in fish oil treated rats. Our study also gives that fish oil is more hypocholesterolaemic than olive oil, which is in accordance with the results reported by others [31]. Many clinical studies have indicated that omega-3 fatty acid content of fish oil is the responsible component [32].

Renal failure is also associated with hyperphosphatemia. The level of inorganic phosphate increases with use of amikacin. This increased level of inorganic phosphate was restored to normal by pre fish oil treatment and coadministered fish oil group suggesting the varied role of omega-3 fatty acid on metabolism. Amikacin also effects liver as the levels of SGOT and SGPT were raised to a high by amikacin. Pre as well as coadministered fish oil treatment again helped in normalizing the levels of these metabolites making it evident that fish oil antagonizes toxicity induced by the drug.

Enzymes are present in biological fluids and various tissues and their altered level is an indication of damage to the particular tissue. Alkaline and acid phosphatases have been shown to be abundant in the kidney [33]. Hence any change in these enzymes levels is an indicative of biochemical alterations in kidney. The decreased level of AlP and AcP in kidney by amikacin, a nephrotoxic agent was restored towards normal in pre and coadministered fish oil treated group giving the beneficial role of omega-3 fatty acids supplementation in restoration of renal membrane structure and function.

Amikacin also induces oxidative stress as evident by significant decrease in activities of CAT, SOD and GST. The role of hyroxical radical in aminoglocoside induced acute renal failure in rats is already established [34]. Fish oil treated groups together with amikacin result in significant rise levels of these antioxidant enzymes thus it helps to ameliorate induced stress. This is in accordance with the results reported by others [35]. The lipid composition of the liver of rats reflects the lipid composition of diet, therefore the lipids of the livers of the rats given fish oil contained higher levels of PUFA. This fact renders the livers of rats fed on fish oil more susceptible to lipid peroxidation and the activity of the antioxidant enzymes may be induced.

Our study also suggests that fish oil results in lipid peroxidation as indicated higher levels of MDA in fish oil receiving animal groups. However the levels of antioxidant enzymes in these groups were also much elevated. This greater activity of antioxidant enzymes may overcome the effects of high lipid peroxidation. This greater activity of antioxidant enzymes may contribute to the hypothesis that fish oil consumption extends lifespan [35]. Less attention has been paid to oleic acid enriched diets. In our study, we found that olive oil could

not bring any profound changes in the levels of antioxidant enzymes. However, it reduced the amikacin induced oxidative stress probably due to oleic acid and tocopherols in it [36].

Concluding the whole study, it seems that omega-3 fatty acid supplementation for 8 days prior to and together with amikacin treatment may be advantageous in combating the number of metabolic and biochemical alterations induced by amikacin. Considering the fact, that oxidation of lipids is the basic process of damages under the action of alpha- and γ-ionizing radiation [37], the received results can be used in the preventive purposes.

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