Effect of dietary n-3 and n-6 fatty acids on tobramycin induced nephrotoxicity

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Post fish oil(n-3 fatty acids) treatment (5mg/kg/day for 12 days) was effective in bringing the reversal of tobramycin (160mg/kg/day,ip for 12 days) induced nephrotoxicity in albino rats as was evident by normal urea, creatinine, cholesterol and inorganic phosphate levels in the serum of the treatment group compared with group receiving tobramycin only. The return of normal levels of alkaline and acid phosphatase in kidney homogenates of post fish oil treatment group also indicated the beneficial effect of dietary n-3 fatty acids(fish oil) more than n-6 fatty acids(olive oil). The results suggest that oral supplements of dietary n-3 fatty acids (fish oil) for nearly two weeks after tobramycin exposure is more beneficial than n-6 fatty acids (olive oil) as it results in reversal of nephrotoxicity induced by tobramycin.

Keywords: Aminoglycosides, Tobramycin, Nebramycin, Nephrotoxicity, n-3 fatty acids, Fish oil, n-6 fatty acids, Olive oil, Urea, Creatinine.

IPC Code: Int. Cl A61K

All aminoglycosides are antimicrobial substances with similar antibacterial spectra and their potential toxicities affect the same organ systems. However, certain subtle differences do exist. Tobramycin (Nebramycin) a six-membered aminocyclitol ring with amino group side chain, is produced by Streptomyces tenebrarius. It is active against all the enterobacteriaceae and is used for treatment of bacterial conjunctivitis, cystic fibrosis and intraabdominal infections. For the treatment of mild to moderate infections, tobramycin is administered in a dose of 3-4.5mg/kg body weight/day, given in three divided doses and can be increased to 5-8 mg/kg/day for serious infections. Aminoglycosides have broad antibacterial spectrum extending from positive aerobic cocci to gram-negative bacilli. About 30% of patients treated with aminoglycosides show some signs of nephrotoxicity. Tobramycin is excreted in urine by glomerular filtration and in normal renal functioning patients, 60% of administered dose is secreted within 6hr. However, some of tobramycin is accumulated in renal cortical cells with half-life of 74hr, making it nephrotoxic. Tobramycin in kidneys causes renal impairment characterized by oliguria, proteinuria and a progressive rise in blood urea and creatinine values together with a decrease in glomerular filtration rate. Tobramycin induced renal damage may be aggravated by concomitant use of other nephrotoxic drugs. Wood et al. demonstrated that vancomycin potentiated tobramycin nephrotoxicity. Similarly, use of cephalotin increased the risk of nephrotoxicity induced by tobramycin. However increased renal toxicity has not been observed when tobramycin was used either with carbenicillins or ticarcillin or cephalosporins. It has also been reported that the ceftriaxone reduces significantly the renal toxicity of Tobramycin in experimental rats.

Fish oil protects against proteinuria in passive Heymann nephritis against cyclosporine nephrotoxicity in rats and in renal transplant recipients. In addition, fish oil has been reported to protect against acetaminophen (paracetamol) induced hepatotoxicity, ethanol induced gastric mucosal injury in rats and in number of inflammatory diseases. Very recently, the beneficial effects of fish oil supplementation in reverting back the nephrotoxicity induced by gentamycin was observed in rats. Keeping this in, the present study has been undertaken to make comparative investigation of impact of fish oil (n-3 fatty acids) and olive oil (n-6 fatty acids) in reducing the biochemical alterations induced by tobramycin. Olive oil was used as a placebo for fish oil because its constituents are similar to, but in much lower concentration. Indeed, olive oil has been used almost exclusively as the placebo in fish oil studies.
**Materials and Methods**

**Animals**—Adult male wistar rats weighing 100-150g were used in all the experiments. Animals were stabilized for 8 days prior to the experiment on standard pellet rat diet and allowed free access to water.

All the chemicals used for 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 (5ml/kg-body weight) to rats with the help of catheter. Tobramycin vials of 2ml, having concentration of tobramycin sulphate as 80 mg in 2ml, obtained from Aristo Pharmaceuticals, India, were given intraperitonially (ip) in one daily dose (160 mg/kg body wt) in volume adjusted to 1 ml with 0.9% saline for 12 days.

**Time dependent effect of tobramycin in rats**—Maximum nephrotoxicity was induced in rats by ip administration of tobramycin (160 mg/kg/day) for 4,6,8,10,12,14 consecutive days. The animals were sacrificed 12 hr 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 12 day treatment with tobramycin.

**Treatment of animals**—The rats were divided into 8 groups of 10 each. All of these groups were treated as follows:

**Group I** (normal) or no treatment group—The rats were given ip injection of normal saline for the last 12 days of the 24 day experiment and were allowed free access to food and water.

**Group II** (control) or tobramycin treated group—The animals were given ip injection of tobramycin for last 12 days of the 24 day experiment to get the measure of maximum nephrotoxicity induced by tobramycin.

**Group III** or oil pre-treatment group—The animals were given oil orally for 12 days and for next 12 days both oil and tobramycin were administered.

**Group IV** or oil co-administration group—The animals were given no treatment for first 12 days and for next 12 days, they were given both oil and tobramycin.

**Group V** or oil post-treatment group—Here the animals were given tobramycin for first 12 days and oil for next 12 days.

Groups III, IV and V were in duplicates, one set being given fish oil and other olive oil. The diet intake was same in all groups.

**Biochemical analysis**—The animals were given pre, post and co-administration of either of the oils with tobramycin injections and were sacrificed 12hr after they received the last treatment. Blood was withdrawn and serum was obtained by centrifugation of blood at 2000 rpm for 10 min. The serum was then deproteinized with 3% TCA in the ratio of 1:3. After incubation for 10 min at room temperature the samples were centrifuged at 1500 rpm for 10 min to obtain protein free serum, which was subjected to various assays.

(i) Quantitative determination of urea by Dam method as described by Fingerhunt et al.\textsuperscript{31} using a reagent kit from Techno. Pharm. Chem; India.

(ii) Creatinine estimation was done by method of Tausky and Bonces\textsuperscript{32} using a reagent Kit obtained from Span diagnostics Ltd., India.

(iii) Estimation of cholesterol content by method of Wybenga and Pillegi\textsuperscript{33} using reagent Kit from Span diagnostics Ltd., India.

(iv) Quantitative determination of inorganic phosphate by the method of Tausky and Shorr\textsuperscript{34}.

(v) SGOT and SGPT levels were determined by method of Reitman and Frankel\textsuperscript{35} using a kit obtained from Span diagnostics Ltd., India.

**Kidney and liver homogenates**—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 min at 20,000 rpm. Supernatant was then subjected to assay of marker enzymes.

**Alkaline phosphatase assay** (Alkpase, E.C.3.1.3.1): The activity of AlkP was determined according to the method of Shah et al.\textsuperscript{36}.

**Acid phosphatase assay** (AcPase, E.C.3.1.3.2): The AcP activity was measured quantitatively by the method of Verjee\textsuperscript{37}.

**Statistical analysis**—Statistical analysis of the data was performed using one way analysis of variance (ANOVA) using SPSS computer software. The difference was considered significant when \(P<0.05\). Values shown as means ±SE for 10 animals.

**Results**

In the present study, rats in the eight experimental groups consumed similar amounts of food. The over-
all magnitude of impaired renal function is reflected by increased level of urea and creatinine in the serum of tobramycin receiving group compared to normal saline treated one. The rise in levels of other serum parameters like inorganic phosphate, cholesterol, SGOT, SGPT, as well as decrease in activities of various markers like AlP and AcP in liver and kidney homogenates also suggest that tobramycin is toxic for various organs.

To see the effect of oils in preventing the induced alterations in renal system by tobramycin, serum urea and creatinine levels were determined. The urea concentrations in serum-measured show that increased levels of urea was decreased in groups that received pre and post treatment of both the oils (Fig.1). This effect was most pronounced in n-6 fatty acid as compared to n-3 fatty acids receiving animal group.

The elevated serum creatinine levels by tobramycin were lowered maximally by the post fish oil treatment (Fig.2). Here pre fish oil treatment also helped to lower the creatinine content in serum, reflecting that renal function is reverting back toward the normal functioning of filtration. The effect of dietary lipids on the cholesterol content of serum is given in Table 1. Animals fed on n-3 fatty acids (fish oil) had significantly lower cholesterol content in serum compared to those fed on n-6 fatty acid diet (olive oil). Further, it is clear that pre fish and post fish oil treatment results in significant decrease of cholesterol levels close to normal, which otherwise was elevated by tobramycin alone.

![Fig. 1—Total urea levels in serum. Values are expressed as mg/100 ml and represent mean ± SE of 10 different animals. *P<0.05 as compared to normal saline treated. Significance between normal and experimental groups(ANOVA).](image1)

![Fig. 2—Total creatinine levels in serum. Value are expressed as mg/100 ml and represent mean ± SE of 10 different animals. **P<0.05, ***P<0.01 as compared to normal saline treated. Significance between normal and experimental groups(ANOVA).](image2)

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>N</th>
<th>C</th>
<th>FI</th>
<th>FII</th>
<th>FIII</th>
<th>OI</th>
<th>OII</th>
<th>OIII</th>
</tr>
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<tbody>
<tr>
<td>Cholesterol (mg/100ml)</td>
<td>29.89</td>
<td>71.55</td>
<td>32.79</td>
<td>64.39</td>
<td>24.66</td>
<td>43.57</td>
<td>132.56</td>
<td>69.13</td>
</tr>
<tr>
<td>Phosphate (mg/ml)</td>
<td>±0.22</td>
<td>±4.86</td>
<td>±1.04</td>
<td>±0.52</td>
<td>±2.03</td>
<td>±7.99</td>
<td>±0.05</td>
<td>±7.37</td>
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<tr>
<td>Inorganic Phosphate (mg/ml)</td>
<td>5.33</td>
<td>20.00</td>
<td>3.61</td>
<td>19.30</td>
<td>3.67</td>
<td>3.17</td>
<td>14.39</td>
<td>3.43</td>
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<tr>
<td>SGOT (U/min/ml)</td>
<td>±0.55</td>
<td>±1.57</td>
<td>±0.30</td>
<td>±0.37</td>
<td>±0.35</td>
<td>±0.39</td>
<td>±2.07</td>
<td>±4.01</td>
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<tr>
<td>SGPT (U/min/ml)</td>
<td>22.14</td>
<td>49.22</td>
<td>43.51</td>
<td>48.83</td>
<td>25.18</td>
<td>37.53</td>
<td>50.77</td>
<td>37.03</td>
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</tbody>
</table>

*P<0.05— as compared to normal treated rats (ANOVA)

N-normal saline treated; C-control or tobramycin treated; FI- pre treated fish oil; FII-coadministration fish oil; FIII-post treated fish oil; OI- pre treated olive oil; OII- coadministration olive oil; OIII- post treated olive oil

Table 1—Circulating levels of various biochemical parameters in serum

[Values are mean± SE of 10 different animals]
It is clear that pre and post fish oil treated group results in normalizing the elevated levels of inorganic phosphate. Here pre and post olive oil was also significant in reducing the level of inorganic phosphate to normal.

One of the common observation was that co-administration of both fish and olive oil together with tobramycin injections resulted in many fold increase of all measured serum parameters like urea, creatinine (Fig.1, 2), cholesterol, inorganic phosphate, SGOT and SGPT.

The serum SGOT and SGPT levels showed a significant increase in tobramycin treated animal groups. Again, in post fish oil treated animals, these high levels of SGOT and SGPT were reduced maximally compared to other oil treated groups. Although this level of SGOT was much higher than normal value but it was still significantly low as compared to very high level in tobramycin treated only.

The effect of tobramycin was also observed in the activities of certain enzymes of kidney and liver. The AIP and AcP level decreased significantly in the homogenates on treatment with tobramycin injections (Table 2). In kidney homogenate, the levels of AIP and AcP were significantly lowered in post fish and post olive oil groups (Table 2). It is also evident that in liver homogenate, AIP level returned towards normal level in post fish as well as in post olive oil treated groups. Similar results were obtained, when level of AcP was determined.

**Discussion**

Tobramycin is nephrotoxic. The present study was designed to determine whether feeding PUFA oil polyunsaturated fatty acid (PUFA; n-3 oil) or MUFA oil monounsaturated fatty acid (MUFA; n-6 oil), helps in reverting the nephrotoxicity induced by tobramycin. This study also determined the effect of the two oils on cholesterol levels in serum or the cardiovascular health. Acute renal failure, induced by tobramycin exposure resulted in an increased serum urea and creatinine levels. The rise of urea and creatinine levels in tobramycin treated animals compared to normal saline treated animals suggest that due to renal injury, glomerular filtration rate (GFR) and reabsorption processes suggest that the present results indicate the beneficial effects of n-3 fatty acids in combating nephrotoxicity, as the levels of urea and creatinine decreased from higher levels in post fish oil treated group (Fig.1, 2). This is in accordance with those reported for gentamycin induced nephrotoxicity.

Hyperphosphatemia usually is related to renal failure. The increased level of inorganic phosphate by tobramycin injection means decrease GFR and increased reabsorption by renal tubules. A decrease in GFR limits the renal excretions of phosphate, and develops hyperphosphatemia. Phosphate in serum is mostly protein bound or complexed with calcium, with some of it free. Phosphate is critical for activity in several important enzyme systems. Hyperphosphatemia, however, does not result in any definable disorder. The abnormalities attributed to hyperphosphatemia are secondary to changes in calcium homeostasis e.g. soft tissue calcification and possible tumoral calcinosis. The increased level of inorganic phosphate by tobramycin was restored to normal in post fish oil treated group (Table 1), suggesting varied effects of n-3 fatty acids on metabolism.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>N</th>
<th>C</th>
<th>F1</th>
<th>F2</th>
<th>FIII</th>
<th>OIl</th>
<th>OII</th>
<th>OIII</th>
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<tr>
<td>kidney AIP</td>
<td>50.2</td>
<td>30.56</td>
<td>38.66</td>
<td>28.18</td>
<td>50.58</td>
<td>46.29</td>
<td>35.47</td>
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<td>±0.09</td>
<td>±0.25</td>
<td>±0.45</td>
<td>±0.04</td>
<td>±0.48</td>
<td>±0.67</td>
<td>±0.64</td>
<td>±0.43</td>
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<td>kidney AcP</td>
<td>113.57</td>
<td>81.20</td>
<td>88.44</td>
<td>81.91</td>
<td>108.34</td>
<td>105.97</td>
<td>78.67</td>
<td>111.33</td>
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<tr>
<td>(nmol/mg/ml)</td>
<td>±0.33</td>
<td>±0.55</td>
<td>±0.47</td>
<td>±0.29</td>
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<td>(nmol/mg/ml)</td>
<td>±0.37</td>
<td>±0.11</td>
<td>±0.39</td>
<td>±0.19</td>
<td>±0.18</td>
<td>±0.03</td>
<td>±0.16</td>
<td>±0.25</td>
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<tr>
<td>liver AcP</td>
<td>39.96</td>
<td>32.97</td>
<td>35.87</td>
<td>31.29</td>
<td>37.97</td>
<td>35.01</td>
<td>30.89</td>
<td>38.57</td>
</tr>
<tr>
<td>(nmol/mg/ml)</td>
<td>±0.08</td>
<td>±0.31</td>
<td>±0.06</td>
<td>±0.23</td>
<td>±0.05</td>
<td>±0.08</td>
<td>±0.32</td>
<td>±0.49</td>
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</table>

*P<0.05 as compared to normal treated rats (ANOVA)

N-normal saline treated; C-control or tobramycin treated; F1- pretreated fish oil; FII-coadministration fish oil; FIII-post treated fish oil; OIl- pretreated fish oil; OII-coadministration olive oil; OIII-post treated olive oil
injections. This was in accordance with the present observations, where animals receiving tobramycin 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 n-3 fatty acids after tobramycin injections helped to some extent in lowering the levels of SGOT and SGPT, which otherwise were increased by tobramycin alone (Table 1).

It seems that the co-administration of n-3 and n-6 fatty acids together with tobramycin exposure, somehow potentiated the damage caused by tobramycin as was evident by many fold increase of serum urea, creatinine (Fig.1, 2) and cholesterol (Table 1). This may be due to the reason that tobramycin is not excreted by the kidneys, thereby increasing the nephrotoxicity. It has already been shown that fish oil does not reduce the renal cortical gentamycin accumulation. Content of gentamicin has been related to the severity of nephrotoxicity. In agreement with this, the increase in all nephrotoxic parameters in coadministration group in the present study may be related to the extent drug uptake by the kidney cortex. Further studies are required to know the actual mechanism involved.

To see any cholesterol lowering effects, the level of cholesterol was determined and it was clear that pre and post fish oil treatment resulted in significant decrease by tobramycin alone (Table 1). Pre olive oil was also found to lower cholesterol to same extent when compared to normal. Lower cholesterol level in plasma of fish oil consuming animals have been reported. In accordance with this, the present comparative study shows that fish oil is better than olive oil in lowering the cholesterol level. Fish oil consumption reduces cholesterol levels in blood and that it is more hypocholesterolaemic than olive oil, although the mechanism by which it is brought about remains unclear. 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. 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.

The decreased activities of AIP and AcP in kidney and liver homogenates returned back maximally towards normal in post fish and post olive oil treated animals, indicating the beneficial use of fish and olive oil in tobramycin exposure.

Fish oil has many benefits, however there is growing concern that habitual intake of large quantities of PUFA may induce carcinogenesis, probably because they are very susceptible to peroxidation and production of free radicals. The production of free radicals has been associated with ageing. However, it has also been reported that PUFA extends the life span of animal models of autoimmune diseases. This is further supported by Valentina and Alonso who reported that PUFA in fish oil may render the liver of rats on fish oil diet more susceptible to lipid peroxidation and the activity of antioxidant enzymes may be induced. However, attempts have been made to define an appropriate proportion of dietary n-3 fatty acids that may show beneficial effect without having side effects like increased clotting time. The present results 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 i.e. aminoglycosides.

**Conclusion**

On the basis of the results, it is suggested the use of fish oil (5 ml/kg/day) for two weeks after tobramycin injection helps in reverting the biochemical alterations induced by the tobramycin i.e. proximal tubular subsegment of kidney damaged by tobramycin is restored structurally and functionally again. It appears likely that the beneficial and preventive effect of omega 3-supplementations on nephrotoxicity is result of a complex interplay between altered inflammatory, hemodynamics and metabolic factors. Whatever the mechanism, dietary omega 3 supplementations may offer a safe therapeutic strategy to diminish nephrotoxicity induced by aminoglycosides.

**References**

6. Simon V K, Mosinger E U & Malerezzy, Pharmacokinetic

