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Original Article

Histological changes of the kidney in gentamycin induced nephrotoxicity and protected role of fish oil.

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Abstract

Background: *The kidneys are one of the vital organs of our body. The kidney contributes to the maintenance of homeostasis by a complex process that involves filtration, active absorption, passive absorption, secretion and the end result is the production of urine. Gentamicin induced acute renal failure is one of the kidney diseases which is very common not only in our country, also in worldwide. Gentamicin is the aminoglycoside of first choice because of its low cost and its reliable activity against all but the most resistant gram-negative aerobes. The fish oil is composed of eicosapentaenoic acid, docosahexaenoic acid & many more, may be one or more of these compounds has got nephro-protective action. This study may ensure the clinicians about safe use of gentamicin among those patients who are resistant to other antibiotics.* ***Objective:*** *To identify the histomorphological changes in the kidney in gentamicin-induced nephrotoxicity of the Long Evans rats and beneficial effect of fish oil against gentamicin-induced nephrotoxicity.* ***Methodology:*** *The study was carried out in the department of Anatomy, Dhaka Medical College (DMC), Dhaka, during the period from February 2005 to January 2006. The experiment was carried out on 40 healthy Long Evans rats. The rats were divided into six groups as Group: A, Group: B, Group: C and Group: D. Each group comprised of 10 (Ten) rats from either sex and was randomly selected.* ***Results:*** *In the present study showed that in normal saline control group of rats all the proximal tubules were normal but most of the proximal tubules showed necrotic changes that is about 77.67% of the tubules were necrotic in gentamicin treated nephrotoxic rats. It was also found that the fish oil treated groups showed less severe form of injury.* ***Conclusion:*** *Histologically kidney of experimental control group (gentamicin treated rats) showed proximal tubular necrosis. In the present study the fish oil showed the partial protection against the nephrotoxicity induced by gentamicin administered during the last 6 days of treatment with fish oil and by ameliorating the histopathological damage.*

Key word: *Histological variations, proximal tubule of kidney of Long Evans rats, gentamycin, fish oil*

Introduction: The kidneys are highly vascular organs of our body. The kidneys eliminate the final products of metabolic activities, excrete the drugs and chemicals and regulate the volume and composition of body fluids (Guyton and Hall, 2006). There are approximately 1-4 million nephrons in each kidney (Junqueira and Carneiro, 2005). They receive approximately 25% of cardiac output. As the kidney concentrates and excretes metabolic waste, chemicals and many drugs, it is often exposed to toxic concentration of these substances and a broad range of drugs, diagnostic agents, and chemicals produces the term “toxic nephropathy”. Although proximal tubular necrosis is a common manifestation of nephrotoxic agents like gentamicin. Proximal tubule dysfunction without tubule necrosis may also be an important aspect of nephrotoxicity (Cronin and Henrich, 1996). Acute renal failure is an important cause of morbidity and mortality in Bangladesh and more than 60% causes are related to medical causes (Rashid et al. 1997) and it is a challenging problem in pediatric medical practice and the mortality remains as high as 50-70% (Rahman et al. 1982; Hoque et al. 1985). Gentamicin causes acute renal failure in 10% to 15% of all cases (Humes et al. 1986). Gentamicin is excreted mainly in urine like most other antibiotics

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and their metabolites (Falco et al. 1969). The range between effective and toxic blood levels is narrow. Gentamicin has a greater affinity for proximal tubular cell and increased accumulation, retention and concentration may be related to its greater nephrotoxicity (Aronoff et al. 1983). In our country fish is the main and cheapest animal protein source (Gupta, 1991). The fish oil contains characteristic long chain polyunsaturated fatty acid (PUFA) or omega-3 as the major nutritional components related to human health and their effect on lowering plasma triglyceride level and increasing beneficial HDL-cholesterol level in man (Jahan et al. 2000). Aukema et al. (1992) also mentioned that, Dietary fish oil alters the fatty acid composition in different organs including the kidneys.

Materials and Methods

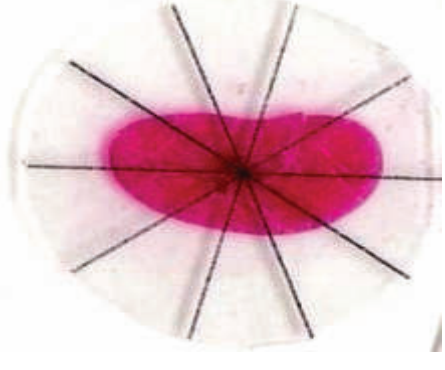
The whole experiment was carried out on a total number of 40 adult Rats, of either sex, of Long Evans Norwegian strain. The animals used in this study weighing between (172-255) gm and the ages were around 7 to 10 weeks. The rats were collected from the animal house of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. They were kept in the animal house of the department of Anatomy, Dhaka Medical College (DMC). For the purpose of identification, each rat was marked as number with coloured permanent marker pen on legs and body after their selection for the experiment. The drugs and chemicals used in the experiments were as follows: Gentamicin (Injection Gentin, Gentamicin BP 80mg/2ml) from opsonin, BD, Fish oil (Omega-3, Fish oil concentrate 1000mg) from Alhambra, USA. , Normal saline (0.9%), Distilled water, Formaldehyde solution. The dose regimen of gentamicin was given as 80mg/kg/day, I/M into the thigh of rat by 1ml plastic disposable insulin syringe. Fish oil as soft gelatin capsule, which contains liquid ingredient in the strength of 1000mg of fish oil concentrate per capsule was used. The dose regimen was given as 5.0ml/kg/day, orally into the stomach. 0.9% normal saline was used only in control group and the dose was 2ml/kg/day (Ali & Bashir, 1994). The rats were divided into three groups. Each group comprised of 10 (Ten) rats from either sex and was randomly selected.

Table I. Grouping of rats, dose of drugs and vehicle, duration of treatment and Sacrifice schedule of the experiment.

Groups	Number of rats(n)	Drugs	Dose	Route of administration
Group A (Normal Control)	10	0.9% Normal Saline	2 ml/kg/day	I M
Group B (Experimental control)	10	Gentamicin	80 mg/kg/day	I M
Group C (Experimental)	10	Fish oil and Gentamicin	5.0 ml/kg/day	Oral
			80 mg/kg/day	IM

Pre treatment Fish oil	Simultaneous treatment	Total duration	Time of sacrifice (24 hours after the last dose)
-	Day 5-day 10	6 days	7th Day
-	Day 5-day 10	6 days	7th Day
Day 1 - day3	Day4-day9	9 days	10th Day
	Day4-day9	6 days	

From each rat only right kidney was taken for histlogical preparation. Identify the total number of normal tubules, total number of tubules having cellular swelling or having vacuolar degeneration, number of tubule having pyknotic or anucleated cell. The number of tubules having mild, moderate and large amount of intraluminal cell debrid were also counted from each field. Standard deviations (SD) and mean of the collected data were calculated for paired student's't' test and comparisons between the groups were made by using ANOVA test (Appendex V).



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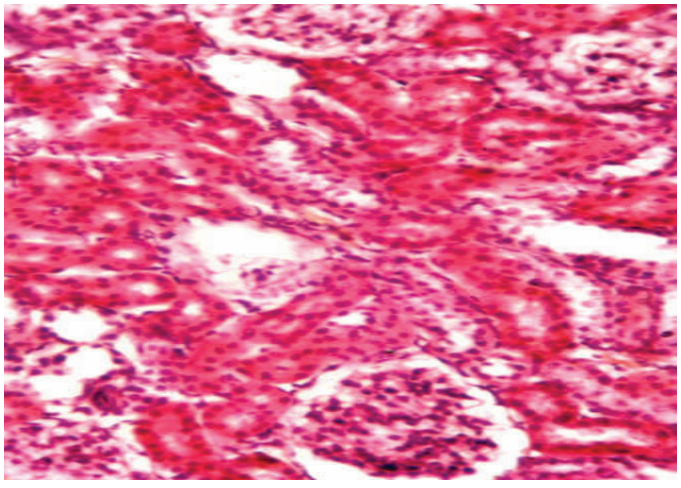

Result

Effects on proximal tubules of the kidney (Table I)

Table I. Percentage incidence of normal tubules and tubules with changes due to gentamicin-induced nephrotoxicity in different group of rats.

Group (n=6)	Normal tubules (Mean)	Tubules with vacular degeneration only (Mean)	Necrosed tubules (Mean)		
			Pyknotic cell containing	Anucleated cell containing	Total
A	100	0	0	0	0
B	17.50	4.83	28.67	49.00	77.67
C	21.33	50.50	18.83	9.33	28.17

Grading of proximal tubular injury based on type and extent of necrosis

Table II. Grading of proximal tubular injury according to the presence of necrosis in different group of rats.

Frequency of rat kidney with different grades of necrosis						
Group (n=6)	**Grade**					
	0	1	2	3	4	5
A	6 (100.0)	0	0	0	0	0
B	0	0	0	0	2 (33.3)	4 (66.7)
C	0	0	1 (16.7)	5 (83.3)	0	0

Values in parenthesis indicates range

Grading system used for assessing proximal tubular injury is as follows (after Houghton et al. 1978; Luft et al. 1976).

Grade 0 : Normal
Grade 1 : Cloudly swelling without necrosis
Grade 2 : Necrosis involving 25% of total cortical area
Grade 3 : Necrosis involving 25%-50% of total cortical area
Grade 4 : Necrosis involving 50%-75% of total cortical area
Grade 5 : Necrosis involving 75%-100% of total cortical area.

Grading of proximal tubular injury based on amount and distribution of intraluminal cell debris (Table III)

Table III. Grading of proximal tubular injury according to the presence of intraluminal cell debris in different group of rats

Frequency of rat kidney with different grades of necrosis				
Group (n=6)	**Grade**			
	0	1+	2+	3+
A	6 (100.0)	0	0	0
B	0	0	2 (33.3)	4 (66.6)
C	0	1 (16.7)	2 (33.3)	3 (50.0)

Values in parenthesis indicates range

The grading system used for assessing proximal tubular injury based on amount and distribution of intraluminal cell debris are as follows (after Zager and Sharma, 1983).

Grade 0 : No debris or trace debris in <10% tubules
Grade 1+ : Small amounts of debris in 10% to 25% of tubules
Grade 2+ : Moderate amounts of debris in 10% to 25% of tubules
Grade 3+ : Moderate to large amounts of debris in >25% tubules and 10 tubular segments per kidney section with > 75% of the entire luminal area filled with debris.

Discussion

In the present study showed that in normal saline control group of rats all the proximal tubules were normal but most of the proximal tubules showed

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necrotic changes that is about 77.67% in gentamicin treated nephrotoxic rats and 28.17% in group C. So the proximal tubular injury was more marked in gentamicin treated groups and fish oil treated groups showed less severe form of injury, this finding correlates with the finding of debnath (2000).

According to the study of Abdel Gayoum et al. (1995) the kidney sections from control group were apparently normal (grade O). However, kidneys of fish oil group had only mild tubular injury (grade I), with most of proximal tubules apparently retaining their normal structures. Ali and Bashir (1994) found in their studies that gentmicin produces proximal renal necrosis (grade 3-4). The histological damage was also less severe (grade 2-3) in fish oil treated groups. Kosek et al (1974) reported that rats receiving the highest gentamicin dose, 40mg/kg/day, showed extensive tubular necrosis. Luft et al. (1976) observed the dose related tubular damage involving primarily the convoluted portion of the proximal tubule.

Houghton et al. (1978) demonstrated that rats receiving gentamicin progressively causes renal proximal tubular necrosis at the end of 10 days. Ali et al. (1992) also found that the drug produced dose-dependent proximal tubular necrosis and seven days after withdrawal of gentamicin, the degree of necrosis was less marked. Well wood et al. (1982) found wide spread necrosis of the proximal tubules in the outer half of the cortex with total loss of all enzyme activity and near the junction of the outer and inner cortex showed gross vacuolar degeneration.

The present study showed that the grading of proximal tubular injury of different groups of rats based on amount and distribution of intraluminal cell debris were 0% in group A ; 66.6% graded as 3+ in group B group ; 50.0% graded as 3+ in group C. Debnath (2000) also found that a higher mean grade of proximal tubular injury based on the amount and distribution of intraluminal cell debris (grade 2+ to 3+) in untreated nephrotoxic rats as compared to normal saline control rats and the other treated groups showed less amount of intraluminal cell debris. That result also signifies the findings of present study.

Conclusion

The present study was carried out on 40 adult Long Evans Rats of both sexes to observe the effect of fish oil on gentamicin - induced nephrotoxicity. Histologically kidney of experimental control group (gentamicin treated rats) showed proximal tubular necrosis. On the other hand, fish oil treated rats showed some protective effect against gentamicin-induced nephroxicity.

The results also indicate that fish oil were less effective in less duration of pretreatment that means the pretreatment duration must be increased to a suitable period for better protection against gentamicin induced nephrotoxicity.

In the present study in histological procedure the myeloid body, mitochondrial structure, tubular brush border could not be observed. Further studies with large sample, with different doses of fish oil for different period of time are recommended. Electron microscopic studies are also recommended.

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