Chapter V

NEPHROTOXICITY PRODUCED IN MICE
BY δ-ENDOTOXIN
Introduction

The vital importance of maintaining the body's "internal environment" has been recognized since the time of Claude Bernard. The kidney is one of the pivotal organs to regulate this "internal environment" by governing the concentrations of other important constituents of the blood and most foreign substances. Several microbial toxins have found to be associated with the nephropathy. Microbial toxin namely ochratoxin A and possibly other ochratoxin have been found to be associated with the endemic porcine nephropathy and several outbreaks in poultry in Denmark as well as in United States [246, 247]. Citrinin also causes kidney damage in experimental animals [248]. The present chapter deals with the nephrotoxic effects of alkali solubilized δ-endotoxin in mice.

Materials

δ-Endotoxin (prepared in our laboratory) Sodium sulphate, Sodium-potassium tartarate, Sodium hydroxide, Copper sulphate, Potassium sulphate, Zinc sulphate, Potassium iodide, Ammonium Chloride, Mercuric chloride (Glaxo Laboratories India).

Methods

Inbred swiss mice of either sex weighing between 20 to 25 g. were maintained on standard pellet food and water ad libitum. The animals were divided into three groups - Group A : Saline control (5.0 ml physiological saline/kg body weight), Group B : δ-endotoxin at a dose level of 70 kitu/kg and Group C : δ-endotoxin at a dose level of 140 kitu/kg. All the treatments were carried out for six weeks, thrice in a week through intraperitoneal route. Blood was collected 24 hours after the last dose. Heparinized blood was used for the estimation of blood urea and non protein nitrogen. Serum was used for determination of total protein, albumin and globulin.
i) Estimation of Non-protein nitrogen

Nitrogen was determined in a portion of protein free blood filtrate by a micro-
Kjeldahl steam distillation method [249-251].

ii) Estimation of whole blood Urea [252]

Urea of the blood was converted into ammonia by digestion with urease. After
removal of the proteins the colour produced by ammonia with Nessler's reagent was
compared with the colour produced under the same condition with a standard
ammonium chloride solution treated with urease.

A 0.1 ml of whole blood was added to a graduated stoppered centrifuge tube
containing 4.5 ml of isotonic sodium sulphate solution (13.2 g anhydrous sodium
sulphate was dissolved in water and the volume was made to one litre). For blank,
4.5 ml isotonic sodium sulphate was taken into another graduated centrifuge tube.
Twenty mg of Jack bean powder was added to each tube. The tubes were
stoppered, mixed and incubated at 37°C for 20 minutes. A 0.2 ml zinc sulphate
solution (10% w/v) and 0.2 ml of 0.5 N sodium hydroxide were added to precipitate
the proteins. The mixture was well mixed by inversion after each addition and was
then centrifuged. Three ml of supernatant fluid (representing 0.06 ml of blood) was
treated with 2 ml distilled water and 1 ml of Nessler's reagent (35 g of potassium
iodide dissolved in 100 ml of water. Saturated mercuric chloride solution was added
to it until a permanent precipitate formed and the volume was made to one litre with
20 percent sodium hydroxide solution. The precipitate was allowed to settle and the
clear supernatant was taken). For standard, 2 ml standard ammonium chloride
solution (= 0.15 mg of urea/ml) was taken into a graduated centrifuge tube. Three
ml water and 1 ml Nessler's reagent were added to it. For standard a blank was
prepared by taking 5 ml of distilled water and 1 ml of Nessler's reagent. Reading of
the standard and experiment were taken at 420 nm. From the reading of standard,
the value of unknown was calculated.
iii) Method for Estimation of Total protein, Albumin and Globulin [253]

Total protein was estimated by Biuret method [253]. A 0.5 ml of serum from each sample was mixed with 9.5 ml of 22.7% sodium sulphate solution (22.7 g sodium sulphate dissolved in 100 ml distilled water) and 2.0 ml was transferred to another test tube marked 'TP' for total protein. The rest was shaken and wait for 10 min. Then clear filtrate was collected by repeated filtration by filter paper (whatman no. 1). A 2.0 ml of final filtrate were transferred to another test tube marked 'Alb' for albumin. A 2.0 ml of sodium sulphate were taken in a third tube marked 'Blank'. A 5.0 ml of biuret reagent (9 g of sodium potassium tartarate was dissolved in 500 ml of 0.2 N sodium hydroxide. Three g of copper sulphate were added and dissolved by stirring. Then 5 g of potassium iodide were added and dissolved and the volume was made up to 1000 ml with 0.2 N sodium hydroxide) were added to each tube of 'TP', 'Alb' and 'Blank'. The content was mixed and warmed at 37°C for 10 min. After cooling the readings were taken at 555 nm. A standard curve was prepared with dry crystalline bovine albumin accordingly. The value of total protein of each sample was calculated directly from the standard curve. The value of albumin was substracted from total protein to get the value of globulin.

iv) Histological studies of Kidney

The animals were sacrificed and the right kidney of each animal were fixed in 4% formal (10 ml of 40% formaldehyde made up to 100 ml with normal saline) for overnight. Then all the kidney tissues were prepared following the usual laboratory procedures [254]. The slides were prepared for microscopical observation to study the structural characteristics of kidney.

Results and Discussion

It is evident from the experiment (Table 5.1 and Figure 5.1) that non-protein nitrogen, blood urea and total serum protein are increased significantly in edotoxin treated animals at a dose level of 140 kitu/kg in comparison to control group. But no significant change was observed at a dose level of 70 kitu/kg. Albumin decreases significantly whereas globulin increases at a dose level of 140 kitu/kg. Histological
studies of kidney of endotoxin treated mice (Figure 5.3, 5.4) showed slight atrophy of glomeruli and tubules with hyalinised material in a few of the tubules which indicate damage of kidney tissue. The histological features are consistent with of minimal lesion glomerulonephritis with proteinuria. Histology of the kidney of control mice (Figure 5.2) showed proliferation of the glomerular cells and the mesangium and tubules with no obvious histological change. Kidney is the most important excretory organ of the body and serves the functions of eliminating the waste products of metabolism. In renal failure, insufficiency of kidney to excrete of blood urea is found. In the same way the other waste products accumulate, particularly nitrogenous substances so that NPN titre of blood increases. Raised values of NPN and urea in blood indicate impaired renal function or acute renal failure. NPN is raised in those conditions in which blood urea is raised [255]. The present study reveals the similar observations and this may be related to renal failure in case of endotoxin treatment. In many diseased states, the serum albumin is reduced and one or more globulin fractions or total globulin level are elevated [256]. A low serum albumin may be due to heavy loss of albumin in urine, decreased formation in liver,

Table 5.1. Non-protein nitrogen, urea, total protein and albumin–globulin level of whole blood in mice after treatment with δ-endotoxin (Means ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Saline Control (15α)</th>
<th>δ-Endotoxin (kituβ/kg)</th>
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<tr>
<td></td>
<td>(15α)</td>
<td>70 (15α)</td>
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<tr>
<td>Non-protein nitrogen (mg %)</td>
<td>30.3 ± 1.1</td>
<td>34.5 ± 1.3</td>
</tr>
<tr>
<td>Urea (mg %)</td>
<td>27.7 ± 1.6</td>
<td>33.3 ± 1.8</td>
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<tr>
<td>Protein (g %)</td>
<td>7.2 ± 0.3</td>
<td>7.8 ± 0.4</td>
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<tr>
<td>Albumin (g %)</td>
<td>4.1 ± 0.2</td>
<td>4.4 ± 0.1</td>
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<tr>
<td>Globulin (g %)</td>
<td>3.1 ± 0.1</td>
<td>3.5 ± 0.2</td>
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<tr>
<td>A/G Ratio</td>
<td>1.3</td>
<td>1.2</td>
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a = No. of animals    b = kilo international toxic unit

** p<0.02             *** p<0.001
Fig. 5.1. Effect of δ-Endotoxin on Non-Protein Nitrogen (NPN), Urea of whole blood and Protein, Albumin, Globulin level of serum in mice
Fig. 5.2. Showing normal histology of kidney with glomerulus and tubules x 600

Fig. 5.3. Showing tubular shrinkage and proliferation of glomerulus cells in this group is apparent x 600
Fig. 5.4. Showing disruption of tubular cells along with degeneration of glomerulus in this group x 600
increased catabolism of proteins or insufficient protein in food [255]. In case of impaired renal functions as seen in nephrotic condition, the heavy loss of albumin in the urine lead to low serum albumin level. A low serum albumin is also found in the severe liver disease due to impaired ability of the liver to form albumin. Most plasma proteins originate in liver and hepatocytes synthesize fibrinogen, albumin and sixty to eighty percent of globulin [257]. The δ-endotoxin causes liver injury (chapter IV) and thereby altered serum albumin. The A/G ratio indicates the balance between total albumin and total globulin and is usually evaluated in relation to the total protein level [257]. A fall in A/G ratio (i.e. decreased albumin and elevated globulin) and increased total serum protein suggest chronic liver disease [255, 258].

On the basis of experimental parameters it may be suggested that alkali solubilized δ-endotoxin is not only hepatotoxic agent but also a nephrotoxic agent though the author feels that further work associated with nephrotoxicity study will be necessary to pinpoint the exact mechanism of action of δ-endotoxin.