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EFFECTS OF LONG TERM ADMINISTRATION OF GENTAMICIN ON CLINICO-PATHOLOGICAL PARAMETERS IN SWISS ALBINO MICE

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ABSTRACT

The effects of long-term intramuscular injection of gentamicin (Gentin® inj., Opsonin, Bangladesh Ltd.) were studied clinico-pathologically on twenty 60 day-old Swiss Albino mice of either sex for 42 days during January to March 2002. All the mice were grouped into four, each consisting of one male and four female mice, of which one group (group A) served as control without giving any treatment while groups B, C and D received recommended (50 mg / kg), medium (75 mg / kg) and double the recommended (100 mg / kg) doses of gentamicin intramuscularly daily for 42 days. All the treated mice produced mild to severe clinical signs, i.e., roughness of the body coat, dullness, depression, anorexia and weakness. At recommended and medium doses the offspring were apparently normal but at double the recommended dose, 10% offspring were died and others were weak and emaciated. No significant gross change was found in lungs, spleen and heart of all the treated groups but the kidney was found soft, flabby and enlarged and the heart was found darker, congested with necrotic foci on the surface. Histopathological changes showed chronic interstitial nephritis in groups B and C following recommended (50 mg / kg) and medium (75 mg / kg) doses while severe glomerulonephritis was observed following double the recommended dose (100 mg / kg) of gentamicin. In liver, histopathological study showed coagulation necrosis following recommended dose, whereas, karyorrhoexis and tissue regeneration were found following medium and double the recommended doses. In lungs, haemorrhage and thickening of interstitial tissues were observed following double the recommended dose of gentamicin in mice. Thus long term administration of higher dose of gentamicin is detrimental to the vital organs.

Key words: Gentamicin, clinical signs, mice, effects, histopathology

INTRODUCTION

Aminoglycoside antibiotics, especially gentamicin, constitute a very important weapon for veterinarians against gram-negative and few gram-positive infections. Like other antibiotics gentamicin is not free from toxic effects both in human being and livestock. Gentamicin can induce ototoxicity and nephrotoxicity (Ali *et al.*, 1992; Reviere,1985) because both organs (ear and kidney) have higher than normal concentration of phospholipids in their cellular matrices. Cationic aminoglycosides are chemically attracted to anionic membrane phospholipids. Among the aminoglycosides, gentamicin preferentially accumulates in renal cortexes resulting nephrotoxicity. Like other amynoglycosides gentamicin also initiate toxicosis by perturbation of renal proximal tubular cell membrane structure (Ali *et al.*, 1992; Beauchamp *et al.*, 1992; Riviere, 1985). The cationic gentamicin is chemically attracted to the anionic phospholipids in the cell membranes of the proximal tubular cells. The renal proximal tubules actively take up gentamicin, concentration in the renal cortex is far greater than those observed concurrently in the serum and other tissues. Studies also have demonstrated a substantially higher renal cortical concentration of gentamicin compared with other tissues in humans (Schentag *et al.*, 1977), rats (Luft *et al.*, 1978), dogs (Cowan *et al.*, 1980), cats (Jernigan *et al.*, 1988b), sheep (Brown *et al.*, 1985), lambs (Weisman *et al.*, 1982), cattle (Haddad *et al.*, 1987), pigs (Riond and Reviere 1988) and birds (Bush *et al.*, 1981). Substantial concentrations are also found in the renal medulla, liver, spleen and lungs in sheep, cattle, birds and rats. Occasionally renal medullary concentration is substantially higher than the concentration found in liver, spleen and lungs (Schentag and Jusko, 1977). Therefore the toxicity of gentamicin must take into account as problem relating to their hazardous effects upon human beings, animals and birds. For this reason an attempt has been made to study the adverse effects of gentamicin, if any, on some clinical, gross and histopathological parameters in mice, a species of significant importance among laboratory animals.

MATERIALS AND METHODS

Experimental design

This study was carried out on twenty 60-day-old Swiss Albino mice of either sex during the period from January to March 2002 in the Department of Pharmacology, BAU, Mymensingh.

The mice were purchased from ICDDR, B, Mohakhali, Dhaka and were kept under close observation in order to acclimatize to the new environment for a period of one week. After acclimatization they were randomly divided into four equal groups comprising one male and four female mice in each and numbered them as groups A, B, C and D and kept group wise in separate cages with feed and water *ad libitum*. Body weight of all groups of mice was recorded before commencement of treatment. Among four groups, one group of mice (group A) was kept as control without giving any treatment while mice of groups B, C and D were treated with gentamicin (Gentin® inj., Opsonin Bangladesh Ltd.) @ 50 mg / kg body weight (0.15 ml / mice), 75 mg / kg body weight (0.225 ml / mice) and 100 mg / kg body weight (0.30 ml / mice) IM respectively for 42 consecutive days. The lowest (50 mg / kg) and highest (100 mg / kg) doses of gentamicin used in this study were the recommended and double the recommended dose respectively.

Clinical examination

The nature and time of onset of adverse effects, mortality if any were recorded carefully after administration of gentamicin for 42 days of treatment period as well as 7 days of post-treatment period.

Gross pathology

At the end of experiment all adult mice were killed by euthanasia with over dosing of ether and subjected to postmortem examination with a view to study the gross morphological and histopathological changes. The gross lesions present in various organs were observed carefully and recorded properly.

Histopathology

Representative tissue samples of liver, kidney, spleen, lungs and heart were collected and fixed in 10% buffered neutral formalin solution for histopathological studies. The well-fixed tissues were placed under running tap water for 24 hours and then they were dehydrated through a series of ascending grades of alcohol (70%, 80%, 95% and 100%). Tissues were cleared by two changes of chloroform and impregnated in paraffin at 58 °C. Finally the tissues were embedded in liquid paraffin. The tissues were sectioned at 4 to 6 μ from paraffin block with the help of rotary microtome and placed in water bath (37-40 °C) for spreading and the sections were taken on grease free clean glass slides and dried in air. The histologic sections were stained with haematoxilin and eosin (H & E) stain following the routine procedure of histopathological studies (Luna, 1968). The stained sections were then permanently mounted in Canada balsam with a cover slip (Luna, 1968).

RESULTS AND DISCUSSION

Adverse effects

Long term administration of gentamicin in recommended (50 mg / kg in group B), medium (75 mg / kg in group C) and double the recommended doses (100 mg / kg in group D) showed mild to severe toxic signs, i.e., roughness of the body coat, depression, anorexia, and weakness. Dantas *et al.* (1997) and Aguiar *et al.* (1997) observed similar findings in dogs as recorded in this study. However, they also found diarrhoea and vomiting following gentamicin administration in dogs. This variation of adverse effects might be due to species variation. All the new born offspring were normal in all groups except group D getting double the recommended dose of gentamicin (100 mg / kg). In this group 10% of the newborn offspring were died 2 days after delivery while others were very weak and emaciated. This finding somewhat contradicts with the report of Lichthorn (1984) who reported death of newborn rabbit following low dose (20 mg / kg) of intramuscular injection of gentamicin during gestation period for one week only.

Gross pathological changes

In group A (control) all vital organs were apparently normal. Kidney became soft, flabby and enlarged in all the treated groups (groups B, C and D) which support the findings of Dantas *et al.* (1997) who reported pallid, swollen and soft kidney following IM injection of gentamicin @ 10 mg / kg body weight in dogs for 10 to 14 days. Liver was darker, congested with necrotic foci on the surface in the treated groups B, C and D. No significant gross change was found in lungs, spleen and heart in any group of mice. Gross pathological changes in liver and kidney of rats following gentamicin administration were also reported by Atef *et al.* (1992) and Kacheva and Krustev (1979).

Histopathological changes

Kidney

There was significant fibrosis in interstitial tissue indicating chronic interstitial nephritis in groups B and C (Fig. 2) following recommended (50 mg / kg) and medium doses (75 mg / kg) of gentamicin respectively. Severe glomerulonephitis was found following administration of high dose (100 mg / kg) of gentamicin in group D (Fig. 1).

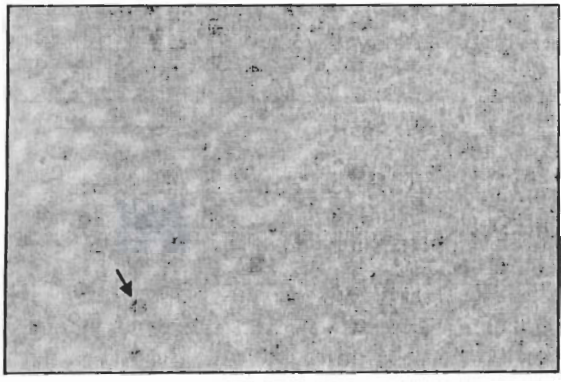

Fig. 1. Glomerulonephritis in kidney in group D; indicated by (→) X 40

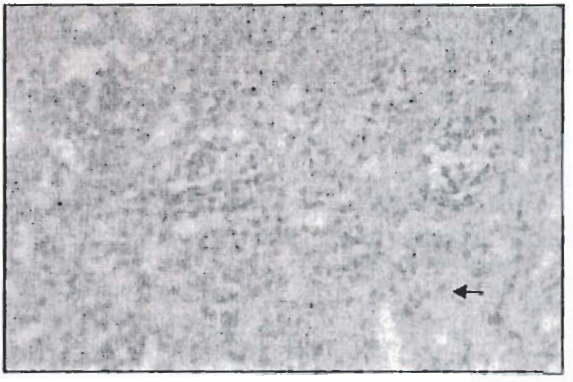

Fig. 2. Chronic interstitial nephritis in goups B and C; indicated by (→) X 40

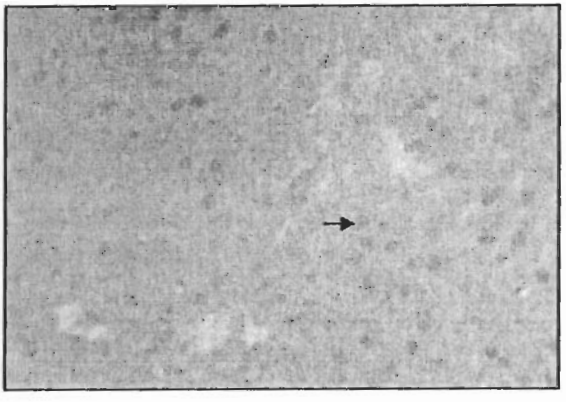

Fig. 3. Karyorrhoexis is liver in group C; indicated by (→) X 40

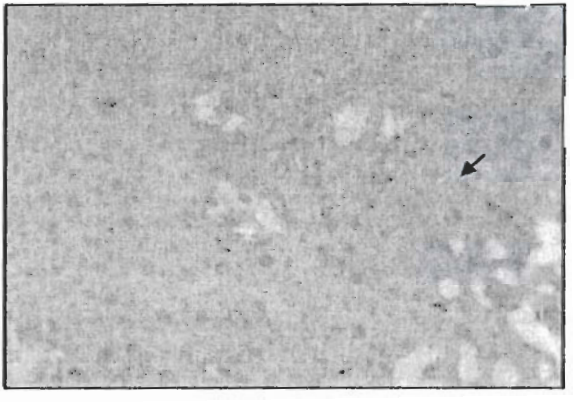

Fig. 4. Tissue regeneration in liver in group C; indicated by (→) X 40

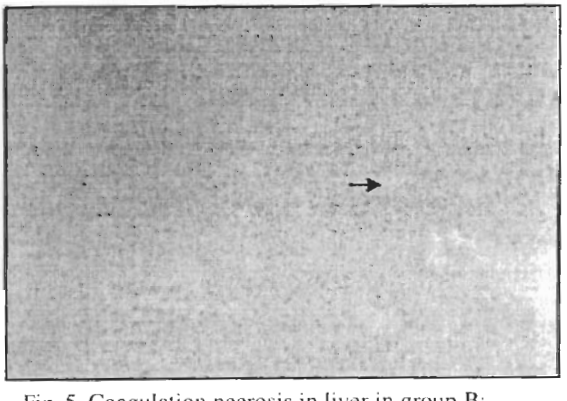

Fig. 5. Coagulation necrosis in liver in group B; indicated by (→) X 10

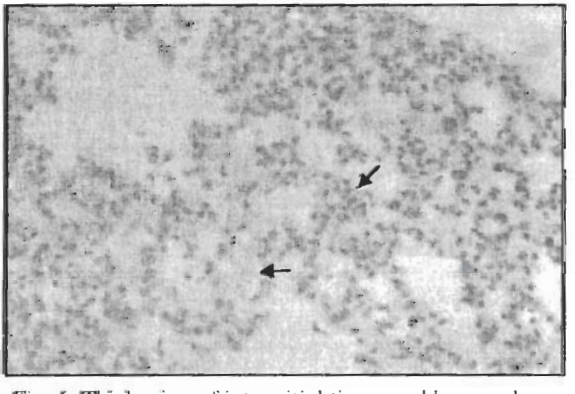

Fig. 6. Thickening of interstitial tissue and haemorrhage in lungs in group D; indicated by (→) X 40

Similarly, Gupta and Verma (1998) reported mild nephrotoxic lesions in guineapigs following IM injection of gentamicin at 4 mg / kg body weight for seven days.

Liver

There was coagulation necrosis in liver (Fig. 5) following recommended dose (50 mg / kg) of gentamicin in group B. Following medium (75 mg / kg) and double the recommended (100 mg / kg) doses in groups C and D, karyorrhoexis (Fig. 3) and tissue regeneration (Fig. 4) were observed. Similar to the present findings, nephrotoxic and hepatotoxic effects of gentamicin were also reported by many other workers like Atef *et al.* (1992), Cronin *et al.* (1980), Dantas *et al.* (1997) and Jernigan *et al.* (1988a). Although exact cause of histopathological changes in liver and kidney could not be fully explained, however, these changes might be due to the toxic effect of gentamicin on kidney and liver during long term administration.

Lungs

Haemorrhage and thickening of interstitial tissues were found following high dose (100 mg / kg) of gentamicin on histopathological study of lung tissue (Fig. 6). This change was found due to thickening of the endothelial cells and partial blockage of the capillaries. However, no significant change was observed following recommended (50 mg / kg) and medium (75 mg / kg) doses of gentamicin. Kacheva and Krustev (1979) found thickening of the alveolar septa and emphysema with many hyperemic blood vessels and stasis in their vicinity and the specific change like nucleolysis of the alveolar cells following exposure to gentamicin aerosol.

Spleen and heart

There was no significant histopathological change in spleen and heart following administration of gentamicin with different doses. Histopathological changes in lungs, spleen and heart were not severe which indicate that these organs are not seriously affected by administration of gentamicin.

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